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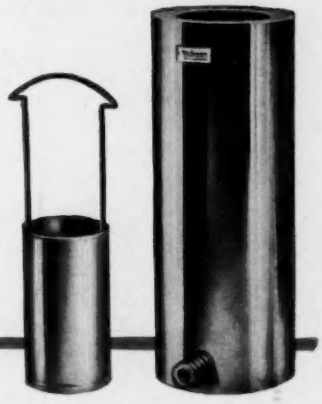

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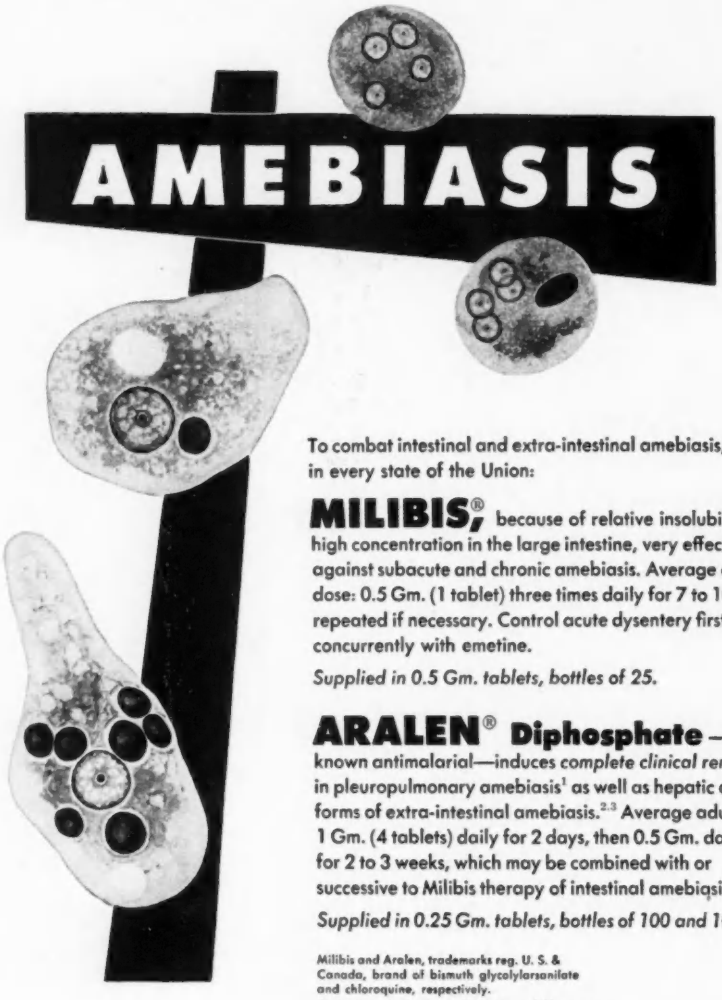

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1. Lindsay, A. E., Gossard, W. H., and Chapman, J. S.: *Dis. Chest*, 20:533, Nov., 1951.
2. Conan, N. J., Jr.: *Am. Jour. Med.*, 6:309, Mar., 1949.
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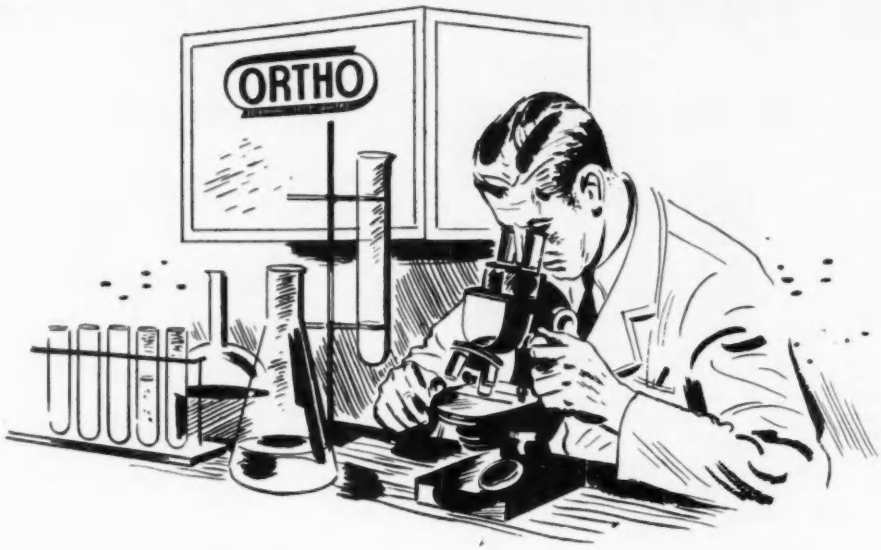

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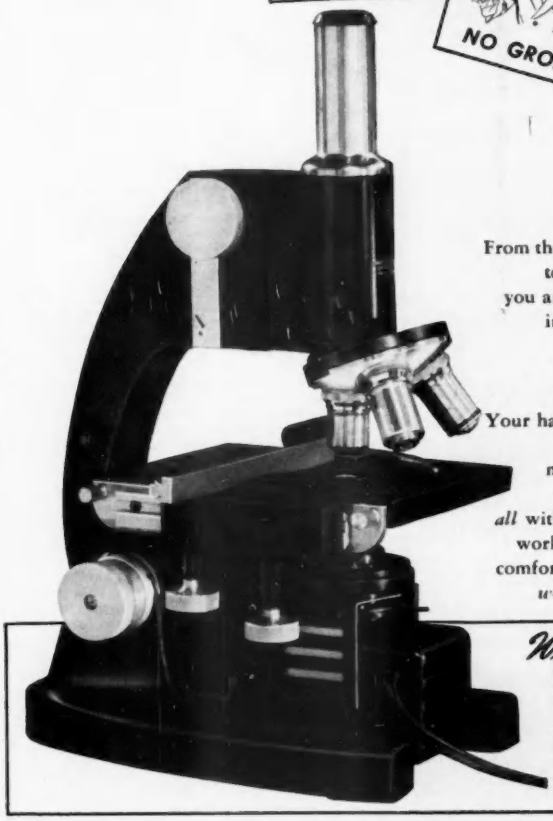

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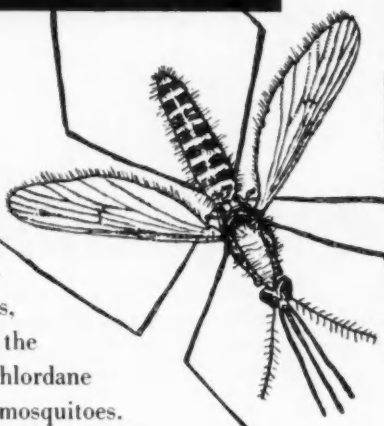

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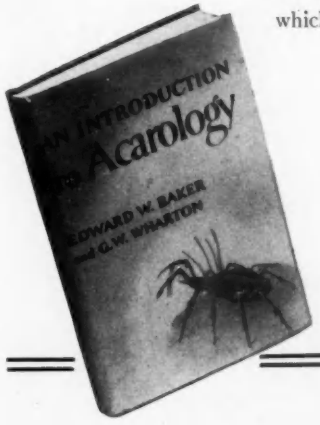

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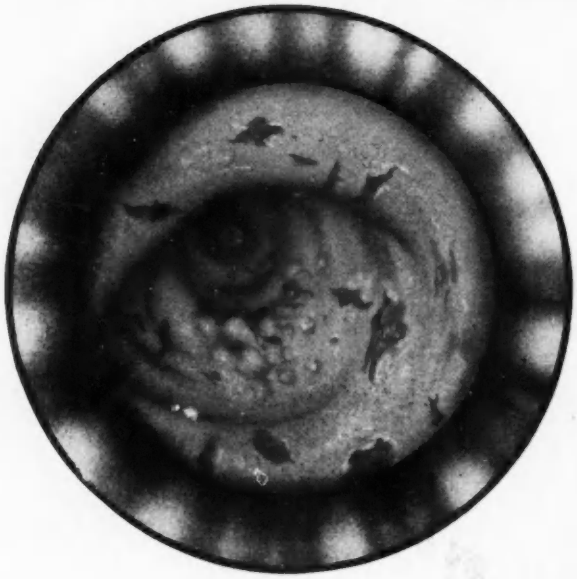

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1. *Dowling, H. F., et al.: Ann. New York Acad. Sc. 53:433 (Sept. 15) 1950.*
2. *Sayer, R. J., et al.: Am. J. M. Sc. 221:256 (March) 1951.*

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When deaths occurred in the untreated group of an experiment the untreated lesion weight was estimated, without bias but less efficiently, as the average of intact untreated groups of technically similar experiments (square root scale). The 95%, 99% and 99.9% limits of expectation for an individual untreated group average was approximated according to

$$\bar{\bar{X}} \pm t \sqrt{\frac{[\Sigma \bar{X}^2 - (\Sigma \bar{X})^2/\bar{N}](N+1)}{N(N-1)}}$$

where $\bar{X}$ is an individual mean (square root), $\bar{\bar{X}}$ is the mean of such means, N is the number of means, and t is Student-Fisher's "t" corresponding to $N - 1$ degrees of freedom and the chosen confidence level. The quantity under the radical is the computational form of the sum of variances of the individual mean and the mean of means. Average lesion weights of (intact) treated groups of similar size falling outside such limits are considered significant at 0.05, 0.01 and 0.001 levels, respectively. Untreated values ($\bar{\bar{X}}$) computed in this manner are shown in table 2 in parentheses.

PUBLIC HEALTH PRACTICE AND POPULATION PRESSURE IN THE TROPICS[1]

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We who are here today have special concern in the well-being of tropical peoples. Furthermore, our interest is not simply theoretical. It is practical. Consequently, it disturbs us to be told that modern health practice in the highly populated tropics may actually be doing harm. It therefore seems appropriate at this time to discuss some aspects of this question about public health and population pressure.

We all realize, I believe, that rapid growth of population in areas already densely peopled creates problems which cannot be solved solely by improving public health. We also know that the world's population is increasing. It approaches 2.5 billions and some demographers assume that if existing trends continue, the total by the year 2000 will exceed four billions. But Notestein (1950) after carefully examining the question, says that the only way to test the assumption is to keep alive until that date! However, death rates more and more frequently fall well below birth rates, a phenomenon that in time could make it impossible to raise the world's living standards. It might even tear them down, in spite of modern technology.

This possibility, remote though it may be, excites much comment in which an apprehension about lower death rates seems to overshadow all other considerations. And although many factors are involved in the lowering of death rates, the effect of controlling disease is so plain that physicians and sanitarians are sometimes held solely responsible for the ills of overpopulation, present and expected. More and more frequently, we read of "dangerous doctors" who are "biological illiterates." "When such men expose millions of their fellow beings to the evils of starvation and disease," we are told, "they must be made aware of the ignorance and the immorality of their acts" (Cook, 1951). Again, sanitarians are "setting the stage for disaster," then, "like Pontius Pilate, washing their hands of the consequences" (Vogt, 1948). We even see the word "fortunate" applied to the estimate that only 12 per cent of the world's population is yet receiving any large proportion of modern health service (Davy, 1951).

So, some imply, and others insist, that we must slow down or stop our efforts to control important diseases in overcrowded tropical areas. Malaria control in British Guiana, for instance, is deprecated because the birth rate has risen while infant mortality has dropped (Cook, 1951; Giglioli, 1951). Sanitarians consider the successful use of residual DDT in that country an outstanding achievement. But others react to it by commenting that "the gadgets of short-term public health can speed the production of festering slums much faster than was possible in the past" (Cook, 1951).

[1] President's Address, American Society of Tropical Medicine, Nov. 1951.

Again, progress in control of communicable disease in Puerto Rico is viewed by some as just another aspect of a "debacle." The new vital tools of public health practice are said to be "creating problems faster than they are solving them." It is concluded that in Puerto Rico "The United States, having provided just enough minor health improvements to insure more births than deaths has unwittingly encouraged the survival of the unfittest to an extent scarcely equalled in the entire civilized world" (Matsner).

Many similar quotations could be given. Clearly, some sociologists and sanitarians are questioning the worthwhileness of disease control in densely peopled countries. Most of the doubters take the view that increased population pressure is due almost entirely to the asepsis, vaccines, environmental sanitation, insecticides and antibiotics of the last hundred years. The fact that improved agriculture may also increase populations is seldom mentioned. But the important point is that we are discussing a trend of thought, a point of view which appears to be gaining adherents and which deserves our careful attention.

The problem is exceedingly complicated and only a few points can be mentioned here. For example, Pearl and Reed in 1920, and Verhulst in 1838 (Pearl, 1927) described a logistic curve which indicates that populations of animals or man do not expand indefinitely without limit. The curve of growth always levels off and becomes asymptotic. And there is no inevitable rate of population growth. The primary forces of natality and mortality will be modified by changes in public policy, public health, food supply, industrial production, status of women, and many other economic, political and social factors. As to food, for example, who knows whether the Nobel prize winner who recently said that today's knowledge of agriculture could produce adequate and nutritious food for four billion people, is right or wrong? Who can estimate the effect in the future of widespread practical photosynthesis and the production of food from nonedible chemicals and crops? The equation of population and food is much more complicated than any present formulation.

However, all calculations as to rate of growth of population and of food supply are beside the main point except, as Notestein (1951) points out, that "they demonstrate a principle that many people are unwilling to face. Sustained population growth, even at a relatively low rate, is not possible for any period of time significant in the history of the human race. In principle, there must come a time at which low death rates could not be maintained without comparably low birth rates. This is an inexorable fact of nature. . . . Death rates must rise or birth rates must fall."

Some commentators on this difficult subject revert to the Malthusian dilemma. You will recall that, at the end of the 18th century, Malthus (1798) concluded, without the help of today's knowledge of public health, soil science, plant genetics and many other pertinent variables, that population growth is determined principally by three factors, namely war, disease and, most of all, food supply. Since he believed that population would always increase faster than food supply, Malthus saw a dilemma; either the death rate must become higher or else human misery must increase.

The use of this word "dilemma" persists in current discussions. Yet actually we are not faced with a dilemma at all. We face a problem. Of course, to avoid increasing population pressure with its concomitant evils, the death rate must rise or the birth rate must fall. But since the latter event would be helpful and not at all disastrous, there can be no true dilemma, no horns of equally undesirable alternatives. We face, not a choice between two evils but, on the contrary, a task well within the scope of man's intelligence and technological potentialities. The evidence available indicates that this problem can be solved. Slowly but definitely it becomes widely apparent that *restraint of birth has equal importance with restraint of death and that the one is no more unnatural or illogical or impossible than the other.*

What are some of the conditions which already are known to lower birth rates? According to Thompson (1948), urban birth rates are lower than rural and the rate decreases as the size of cities increases. Again, the higher the economic status the lower the birth rate, whether rural or urban; the more skilled the father in his trade, the lower the birth rate; and the more schooling possessed by the mother, the lower the birth rate. These are general findings which indicate that birth rates fall, albeit gradually, as standards of living, education and health rise. In such observations it has been reported that planned parenthood has been the chief cause of lower birth rates. Such factors as ambition to get ahead, desire for more comfort and greater freedom, efforts to maintain higher family standards of living, hopes that because they are fewer, the children will be better educated and will have greater opportunities to succeed, all these motives increase as community welfare rises to higher levels. All tend to lower the birth rate.

These benefits of better education and more productive agriculture and industry have been obvious social needs for a long time. But they cannot be much developed in a community where disease is unchecked. Also, it is the absence of public health, with resultant high death rates, which has fostered the growth of social systems responsible for high birth rates. For these reasons, among others, the problem of population densities will not be solved by neglecting to suppress disease at home or abroad, by utilizing deliberately those forces which heedlessly destroy human lives and cultural institutions (Russell, 1951). Rather, let modern health practice be a major activity around which other public services will combine their efforts to change the social fabric for the common good. Experience teaches that without reasonable public health there will not be much planned parenthood or development of public welfare.

But we are told that we should reduce our medical and public health practice in crowded tropical areas until that essential force of public opinion can be developed which will bring birth rates down to equality with death rates. Attempt economic development, improved agriculture and mass education first; postpone energetic practice of public health until there is a widespread desire for family limitation. Some recent authors seem to say, "Do not check diseases like malaria with modern weapons in such places as India because you will upset nature's balanced human ecology. You will increase population pressure, thereby adding

to human misery. For the present, let disease keep down the population much as it has in the past. Then, while pestilence kills off surplus children, we will raise the economic level, develop education, increase the food supply, and implant in the people a desire for limited families. Afterwards, it will be safe to introduce the newer public health gadgets."

Such a view seems utterly inconsequential and without foundation in fact or morals. This crude policy has not a single success on record. And how can one properly speak of a "balanced human ecology" in disease-ridden countries where for centuries the average farmer has not had enough to eat! Moreover, anyone attempting today to decide what populations should be deprived of modern public health would certainly "create problems faster than he could solve them."

The needs are apparent. Huxley (Cook, 1951), for example, and many others have emphasized the importance of a positive population policy for the world as a whole and for each nation in it. Future generations must not be born into increasing misery. The human race must not be allowed to suffer genetic degeneration. We realize this but we do not agree that the answer lies in a lessening of our efforts. On the contrary, our task now, as physicians and sanitarians, is to expand our practice and to organize team work with other scientists and educators in tropical areas that we may devise and operate logical population practice. A major and specific effort should be made to bring together geneticist, mathematician and physiologist, with medical and sanitary scientists, for the advancement of the much neglected study of human heredity and population growth.

Too often the phrase "population policy" connotes simply some contrivance or other for stimulating migration or for encouraging or discouraging childbearing. While fertility regulation in most cases and migration in some are indeed basic, yet, as Myrdal (1947) points out so clearly, a population policy should permeate the whole fabric of social life. Our help is required in organizing practical programs which will combine the techniques that influence birth and death into a logical and balanced community effort. *Man, who has so brilliantly devised measures for restraining death, may be expected to have similar success in regulating birth.* And, it is reasonable to believe that we can bring about the necessary social changes more quickly and more thoroughly where our control of disease is most effective, where our specialized practice is best developed. Of course, one cannot expect a perfect timing of effort which would completely avoid population pressure. But the forces concerned are controllable.

Finally, the world needs today not more disease but more vision! Why emphasize the limiting value of death? How infinitely more intelligent is the idea that family size should be such as will permit economic opportunity, health and social well-being for all!

So, we should not be deceived. We cannot help by withholding our aid! Rather let us maintain our ideals of service. *Let us lead the way with balanced medical and health care programs, designed for sustained public welfare.* Keeping better health as our own main objective, let us integrate our activities with

those of agriculturists, demographers, economists, educators, political and religious leaders, and social scientists. Let us join in planning a social reorientation which will result not in bigger populations but in healthier communities. In this way we shall accelerate progress towards higher planes of health and living. And thus we may play our chosen parts, confident that we are contributing to the welfare of mankind throughout the world.

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COMPLEMENT FIXATION TESTS AS AN AID IN THE DIFFERENTIAL DIAGNOSIS OF EXTRA-INTESTINAL AMEBIASIS[1]

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Extra-intestinal infections with *Endamoeba histolytica* frequently offer difficulties in differential diagnosis. The most desirable serological aid would be a test in which the results are positive in extra-intestinal infections, but negative in cases of only intestinal infection. Though by no means diagnostic, the test employed in the present series approaches the fulfillment of these criteria.

Numerous antigens and technics for complement fixation tests in amebiasis have been used by others during the last 37 years. A detailed review of the results obtained until 1940 was made by Magath and Meleney (1940). Most of these antigens have been made by methods that differed considerably from the one utilized in preparing the commercial antigen used in the present study. The latter was a saline extract of *E. histolytica* grown in culture with a single strain of bacteria.

Craig (1927) was the first to report the results of antigens prepared from *E. histolytica* grown on artificial media. He used absolute alcohol to extract young cultures of the organisms. In his hands (1937) this type of antigen, using a human hemolytic system, gave positive complement fixation tests over an 8 year period in 175 patients on whom stool examinations were performed. The stools of 157 of these patients or 90 per cent contained *E. histolytica*. His antigen was sufficiently sensitive to give positive results in carriers; however, he found that in cases of amebic dysentery the test became negative within 2 weeks following the disappearance of amebae from the stools and the cessation of treatment if the infection had been eliminated.

A further development was reported by Rees and his associates (1942). By removing cysts from contaminating bacteria, they succeeded in growing the amebae in the presence of a single strain of a pleomorphic bacillus which, for lack of definitive classification, they termed organism "t". The amebae were washed and then frozen once with dry ice. After thawing, the suspension was maintained over night at 10°C. It was sedimented in an angle centrifuge for 15 minutes at 15,000 r.p.m. and the clear supernate was used as the antigen for testing two groups of sera. One consisted of 66 sera derived from 34 sources. There was agreement between the results of the tests and the presence or absence of *E. histolytica* in the stools in at least 70 per cent of the cases. The other series, consisting of 35 cases from a single institution where reliable diagnostic

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

procedures were employed, gave a corresponding agreement in 97 per cent of the cases.

Terry and Bozicevich (1948) later prepared an antigen from *E. histolytica* and organism "t" grown in dialyzing tubes immersed in the fluid portion of the medium described by Rees, Bozicevich, Reardon and Daft (1944). Complement fixation tests were performed on the sera from 15 cases of various grades of amebic hepatitis and liver abscess. They were able to obtain sera from 13 of these patients before therapy was started. All but one of the 13 gave positive results. No false positive reactions were obtained.

Hynson, Westcott and Dunning, Inc., prepared an antigen by a method essentially similar to that of Rees and his associates (4) except for the fact that the culture was grown in a cellophane sack, thus keeping it separated from the basic media (Brewer). Standardization of the complement fixation procedure was reported by Kent and Rein (1946). This antigen and test procedure were evaluated in studies by Hussey and Brown (1950) with results in close agreement with those obtained by Kent. The former obtained negative tests on 121 out of 124 patients (97.6 per cent) who had asymptomatic or varying degrees of symptomatic intestinal amebiasis. Approximately the same results were obtained in a group of 116 noninfected individuals. However the sera from 14 out of 17 patients (82 per cent) who had unquestioned or probable hepatic amebiasis gave positive tests. They concluded that the fact that the test is usually negative in intestinal amebiasis makes it an especially valuable aid in the differential diagnosis of hepatic amebiasis as the commonly concurrent intestinal amebiasis would rarely be responsible for a positive test. The same antigen and test procedure were employed in the present evaluation.

PROCEDURE

The complement fixation test employed was that of Kent, Bukantz and Rein (1946) using sheep cells and three 50 per cent units of complement with a fixation period of 4 hours in the refrigerator. As stated before, the *E. histolytica* antigen was prepared by Hynson, Westcott and Dunning. Lyophilized guinea pig complement and rabbit anti-sheep hemolysin were obtained from Sharpe and Dohme. Red blood cells were obtained from sheep maintained at this hospital. The blood was drawn aseptically into Alsever's solution and kept at about 4°C. in accordance with the technic of Bukantz, Rein and Kent (1946). The cells were washed three times with buffered saline just before using. The human sera were removed from the clot less than 24 hours after the patients were bled. A test was considered positive if there was 50 per cent hemolysis or less in the tube containing a 1:2 or higher dilution of the patient's serum. Titrations of the sera to 1:256 were at first routinely performed; but in the later phases of the study, after the antigen was no longer available commercially, many of the sera were tested only at a 1:2 dilution in order to conserve antigen.

Stool examinations were made by Mr. Loyal S. Suter, Protozoologist at this hospital, utilizing the zinc flotation and direct methods and in some cases an

iron hematoxylin stain. Sputum and pus examinations, when made, were by direct smear.

SOURCES OF SERA

Complement fixation tests were performed on sera from a total of 2592 individuals. These comprised 2329 patients in this general hospital and 263 employees of the hospital as presumably normal controls. For the evaluation of the results of the serological tests, the patients were divided into 3 main groups: a) those with extra-intestinal amebiasis either with or without evidence of intestinal infections, b) those with intestinal involvement only and c) those with equivocal or no evidence of amebiasis. This classification was based on a review of all clinical and laboratory findings other than the results of the complement fixation tests.

In the 22 cases of extra-intestinal amebiasis, the factors considered in arriving at the diagnosis included: the presence of a liver or lung abscess that yielded typical reddish brown pus; the demonstration by X-ray of an elevated and irregular diaphragm with adhesions to the liver and decreased motility; lung involvement as evidenced by X-ray findings of increased density or consolidation associated with liver disease; characteristic improvement of liver and lung conditions after anti-amebic treatment.

E. histolytica trophozoites or cysts were demonstrated either in the stools, sputum or abscess drainage in 14 of the 22 cases. Tissue sections available on 5 cases revealed no parasites. The duration of symptoms before admission to the hospital varied from 3 days to 14 years.

There were 187 cases of intestinal amebiasis without evidence of further involvement. *E. histolytica* trophozoites or cysts were demonstrated in the stools of all of these patients except for 2 in whom characteristic lesions were observed on proctoscopic examination. The group of 2383 individuals who were classified as having equivocal or no evidence of amebiasis comprised the control series and a large number of patients whose sera were sent to the laboratory for other diagnostic procedures. Stool examinations were not performed on most of these; however, no patients were included in this group who yielded positive stool examinations. Some of the patients had conditions that may have been due to amebiasis, but the evidence was not sufficient to establish the diagnosis.

RESULTS

None of the sera from the 263 employees of the hospital used as normal controls gave positive complement fixation tests.

Positive tests were obtained with the sera from 19 of the 22 patients with extra-intestinal involvement as shown in Table 1. The titers ranged from 1:2 to 1:256. When this group of 22 cases was further classified into subgroups on the basis of the location of the lesions, it was found that there were 5 cases of liver abscess only, 4 cases of liver involvement without demonstrated abscess and 13 cases in which both the liver and lungs were involved. One case in each of these subgroups gave negative complement fixation tests. Frank abscess of

the liver, either with or without lung involvement, was demonstrated on laparotomy, by aspiration or by X-ray examination in 10 of the 22 cases of extra-intestinal amebiasis. Of these, 9 gave positive complement fixation tests. The single negative case was admitted to the hospital 5 weeks after the first symptoms were noted.

In the group of 22 patients with extra-intestinal infections were 5 in whom symptoms related to liver disease were sudden, acute, and of short duration before admission to the hospital and without a previous history of diarrhea. Two of these gave positive and 3 gave negative complement fixation tests throughout their stay in the hospital. One of the latter gave a negative test as late as the 10th week. In one case with sudden, acute symptoms the sera gave consistently negative tests until the 8th week of illness, but there was a significant rise in titer by the 11th week. The earliest positive test in any of these cases of liver involvement was obtained one month after onset of symptoms. It is difficult to evaluate the serologic response to therapy as there was no prompt drop in titer and as the patients were discharged as soon as this was warranted by clinical improvement. However, in the few cases that were followed for 6 months or longer there was a gradual decrease in titer.

An analysis was made to determine whether there was a relation between the titers obtained and the duration or clinical severity of the extra-intestinal infections. There was no correlation between the titers and the duration of infection as indicated by a history of suggestive symptoms or specific therapy. Of the cases with a duration of 6 months or less, there were 7 with titers greater than 16 and there were 7 with titers of 16 or less. In the group with histories indicating amebic infections extending from 4 to 14 years, 3 had titers above 16 and 5 had titers of 16 or less. On the other hand, there was a definite correlation between the titers and the severity of the infections. The sera that had the highest titers were from the patients who had the most extensive tissue involvement.

The significance of a positive test could be demonstrated if the series of patients had been a completely unselected group. The series of 2329 patients, excluding the control group, approximates this, as most of the sera sent to the laboratory for any test during the period of the study were also tested for their reactions with the amebiasis antigen. Therefore most of the patients on whose sera requests were made for amebiasis complement fixation tests probably would have been included in the series at the time of requests for other tests. Though the series is not ideal in this respect, the available figures furnish a basis for an approximate evaluation. Among the sera from the total of 2329 patients, 67 (3 per cent) gave positive tests. Of the latter, 19 (28 per cent) were from patients with extra-intestinal amebiasis, 32 (48 per cent) from patients with intestinal amebiasis only, and 16 (24 per cent) from those with equivocal or no evidence of amebiasis. If a similar analysis is made of only the positive sera from the 51 cases of all types of amebiasis, it is found that 19, or 37 per cent, were from cases of extra-intestinal amebiasis.

Two complement fixation tests for syphilis were performed on sera from all

the individuals included in the study. Both tests employed the Kolmer technic, one with the Kolmer tissue extract antigen and the other with the cardiolipin antigen. The results are given in Table 1. A comparison was made of the results of these tests in the groups of cases with positive and with negative complement fixation tests for amebiasis. There was no statistically significant difference in the incidence of positive results given by the Kolmer and/or cardiolipin tests (Diff./$\sigma_{Diff.}$ = 1.59), the Kolmer tests (Diff./$\sigma_{Diff.}$ = 1.62) or the cardiolipin tests (Diff./$\sigma_{Diff.}$ = 1.60). In this respect, the only group based on diagnosis that approached significance was that composed of patients with intestinal amebiasis only. Here the difference of the incidence divided by the standard error of the difference equalled 2.30 for either or both tests for syphilis and also for the cardiolipin test alone. The corresponding value for the Kolmer test was,

TABLE 1

Results of complement fixation tests for amebiasis and syphilis

DIAGNOSIS	NO.	AMEBIASIS CF POSITIVE								AMEBIASIS CF NEGATIVE							
		No.	%	K and/or C* Positive		K and C Positive		K+ C− No.	C+ K− No.	No.	%	K and/or C Positive		K and C Positive		K+ C− No.	C+ K− No.
				No.	%	No.	%					No.	%	No.	%		
Extra-Intestinal Amebiasis	22	19	86	1	5	1	5	0	0	3	14	0	0	0	0	0	0
Intestinal Amebiasis, only	187	32	17	6	19	4	13	0	2	155	83	4	3	4	3	0	0
Equivocal or No Evidence for Amebiasis	2120	16	1	3	19	3	19	0	0	2104	99	183	9	122	6	2	59
Controls	263	0	0	0	0	0	0	0	0	263	100	15	6	10	4	0	5
Total	2592	67	3	10	15	8	12	0	2	2525	97	202	8	136	5	2	64

* K = Kolmer Complement Fixation Test; C = Cardiolipin Complement Fixation Test (Kolmer Technic)

1.67. These figures indicate that there was 1 chance in 46 and 1 in 11, respectively that this was due to chance selection of cases. As it is generally accepted that a difference is significant only when it equals or exceeds three times its standard error, no statistically significant relationship was found between the results of complement fixation tests for amebiasis and syphilis. However, the above analysis suggests that such a relationship existed and that in a larger series the difference probably would be significant. From the available data it is impossible to determine whether the indicated difference was due to a small percentage of false positive tests for syphilis in the presence of amebiasis or for amebiasis in the presence of syphilis or whether there was some extraneous factor, such as economic or sociological, that tended to increase the incidence of both diseases within certain groups of the population.

The incidence of positive cardiolipin tests in the presence of negative Kolmer tests was identical in the groups of cases with positive and with negative com-

plement fixation tests for amebiasis (3.0 ± 1.4 per cent and 2.5 ± 0.2 per cent). The incidence of positive cardiolipin with negative Kolmer tests was also identical in the total series of 2592 individuals in this study and in another series of 15,019 patients in this hospital previously reported (McDearman, 1949, 2.55 ± 0.21 per cent and 2.64 ± 0.09 per cent).

DISCUSSION

The results of this study and that of Hussey and Brown (1950), using identical antigen, are in close accord in extra-intestinal amebiasis, positive results having been obtained in 86 per cent and 82 per cent of the cases, respectively. There was greater divergence in the findings in the two groups of intestinal amebiasis. Hussey and Brown obtained positive complement fixation tests in 2.4 per cent of the cases, while in the present series such results were found in 15 per cent of the cases that had *E. histolytica* trophozoites or cysts in the stools.

Both studies clearly indicate the value of complement fixation tests with an antigen of this type in the differential diagnosis of extra-intestinal amebiasis. The comparative lack of sensitivity of the test in the intestinal form of the disease enhances its value for this purpose. However, such an antigen has a limited applicability as it is of value almost exclusively in cases of suspected extra-intestinal amebiasis. That antibodies are present in most cases of intestinal amebiasis is indicated by the work of numerous investigators including Craig (1927) who obtained positive reactions with the sera from 90 per cent of the patients whose stools contained *E. histolytica*. The most desirable antigen, therefore, would seem to be one that when used at a high concentration would detect the low level of antibodies present in intestinal amebiasis. Such an antigen probably would be effective also in the differential diagnosis of extra-intestinal conditions when used at a dilution that would eliminate most reactions due to intestinal infections alone. This dual role of the antigen used at two dilutions would add greatly to the scope of its usefulness.

SUMMARY

Complement fixation tests were performed on 2592 individuals in a general hospital using a commercially prepared *Endamoeba histolytica* antigen. Positive reactions were obtained with the sera from 86 per cent of the cases of extra-intestinal amebiasis, 15 per cent of the cases of intestinal amebiasis only and 0.7 per cent of the cases that had equivocal or no evidence of amebiasis.

The type of antigen employed is of especial value, therefore, in drawing attention to the possible presence of *E. histolytica* infections other than intestinal and as an aid in the differential diagnosis of hepatic and pulmonary conditions.

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OBSERVATIONS ON EXPERIMENTAL CHAGAS' DISEASE IN DOGS[1]

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As a part of an investigation of procedures for the preclinical evaluation of compounds active against *Trypanosoma cruzi*, a study of the course of experimental Chagas' disease in dogs seemed desirable. Confirmation of chemotherapeutic activity and drug tolerance in an animal more closely resembling man in its metabolism and response to medication was considered a necessary adjunct to information gained in screening tests in mice (1–3).

The susceptibility of dogs to infection with *T. cruzi* was established by Chagas and reported in his earliest publications on American trypanosomiasis (4, 5). In the years that followed experimental infections in dogs were used in studies on complement fixation (6–13), neurotropism (14–26), diagnostic isolation (27–30), pathology (31–39), hematology (40), electrocardiography (41–44) and intradermal reaction (45).

It was apparent from the above literature that considerable variation might be expected for different strains of *T. cruzi* and that the probable behavior of any given strain could not be anticipated, except in a very general way.

The present report deals, therefore, with the variations in the incubation period, patency, and pathogenesis of experimental Chagas' disease in dogs as influenced by age and sex of host, route of inoculation, and strain of parasite. Certain observations on serological reactions and chemotherapeutic testing are also included.

MATERIALS

Strains of T. cruzi. Infections in dogs have been produced by inoculation with the following 3 strains of *Trypanosoma cruzi* Chagas, 1909:

Strain "A", was obtained in April, 1947, from Dr. Robert J. Schnitzer of the Chemotherapeutic Laboratory of Hoffman-LaRoche, Inc., Nutley, N. J. The original source of this strain is unknown. It came to Hoffman-LaRoche in August 1942 from the Army Medical School, through the American Type Culture Collection, and its virulence was enhanced by rapid passage through 3 week old Webster mice (46). Since September 1947 it has been maintained in culture.

Strain "B" ("Brazil"), was obtained in November, 1946, from Dr. Theodore S. Hauschka of the Institute for Cancer Research of Lankenau Hospital, Philadelphia. This strain, isolated from a Brazilian patient in 1942, and maintained in culture at the National Institutes of Health, was obtained by Dr. Hauschka in December 1945.

Strain "W" ("R" of Hauschka (47), "Wellcome BH" of vonBrand, *et al.* (48), WBH of Hauschka, *et al.* (49), "Reichenow" of Goodwin *et al.* (50)) was

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obtained from Dr. Hauschka. This strain, isolated from a Brazilian patient in 1926, has been maintained by continuous serial syringe passage in mice, by Reichenow at Hamburg until 1936, then by Hoare at London until 1947, at which time Hauschka obtained it.

These three strains of *T. cruzi* will be referred to in the text below simply as "A", "B", and "W". The "Culbertson" strain, used as test antigen in the agglutination reactions, was also obtained from Dr. Hauschka.

Experimental Animals. Although all of the dogs used were mongrels, some attempts were made to minimize the variables other than genetic and to standardize their handling. Whenever pregnant females were discovered among the dogs received at the laboratory they were isolated and their litters kept intact. The puppies were infected when 10 weeks old. The adult dogs were short-haired animals of apparent hound-terrier ancestry, selected for uniformity in size. All of the animals used were dewormed (with tetrachlorethylene) and given distemper prophylaxis (Green distemperoid virus) prior to infection.

METHODS

Maintenance of T. cruzi. Although "A" and "B" were received in infected mice and were maintained for various periods by syringe passage, the method of greatest convenience in providing inoculum for tests in mice (2) was growth of the organisms in culture.

The medium first used was that devised by Johnson (51), comprising an agar base containing beef infusion and Neopeptone (Difco) with 10% defibrinated rabbit blood, overlaid with Locke's solution. This medium was later modified by use of veal instead of beef infusion and still later by substituting Bacto brain heart infusion agar with P.A.B. as the base, to which the blood and Locke's solution were added as above. No differences in the abundance or rate of growth of *T. cruzi* were observed when these modifications were made. Strains "A" and "B" were maintained by serial bi-weekly transfer in this medium.

In preparing cultures of "Culbertson" strain for use in agglutination tests, the method recommended by Hauschka *et al.* (49) was followed. In this procedure the Locke's overlay stood in contact with the blood agar base for 48 hours and then was distributed into culture tubes as a liquid medium. "W" was maintained by serial syringe passage in mice (Webster) at weekly intervals, throughout this work.

Testing in Dogs. Dogs and puppies, previously dewormed and given distemper prophylaxis, were infected by subcutaneous, intravenous or intraperitoneal injection of either one hundred million cultural forms of *T. cruzi* ("A" or "B") or 500,000 trypanosomes ("W"), in infected mouse blood, per kilogram of body weight.

The bloods of the infected dogs were examined daily throughout the prepatent and patent period and daily trypanosome counts on wet preparations were made. In making the trypanosome counts a large drop of blood, from one of the venules of the ear, was mixed with a small drop of sodium citrate solution, covered and examined under high power (10× ocular, 43× objective); 200 high power fields

were examined each day as long as the animals remained negative. When they became positive this number was reduced to 50 and if they again became negative at any time the number of fields examined was increased.

In some cases slide agglutination reactions were performed according to the method of Hauschka *et al.* (49), in an attempt to determine the status of the infection during the aparasitemic phase. In the heterophile antibody test the method of Davidsohn was followed (52). Animals which died or were sacrificed were autopsied, and representative tissues were fixed in 10% formalin. Paraffin sections, stained in hemalum and eosin, were used for histopathological examinations.

OBSERVATIONS

The course of experimental Chagas' disease in dogs has been observed in a total of 60 dogs, 18 of which were adults, the remaining 42 being puppies 10

TABLE 1

Prepatent periods of experimental Chagas' disease in female dogs, infected by subcutaneous and intraperitoneal inoculation of T. cruzi ("B") culture

AGE OF DOGS AND TIME OF INFECTION	LITTER OR RELATIONSHIP	LENGTH OF PREPATENT PERIOD (DAYS)	
		Subcutaneous inoculation	Intraperitoneal inoculation
Juveniles infected, June 16, 1949	Litter 2	25	28
	Litter 3	36	42
Adults infected, March 4, 1950	All unrelated	21	26
		24	30
Means		26.5	31.5

weeks old when infected. Five of these animals (4 of them puppies) were infected with "A", 6 (all adults) with "W" and the remaining 49 (of which 11 were adults) with "B".

Prepatent Period. In 3 puppies infected intravenously with "A", parasites first appeared in the blood in 10 or 11 days. One puppy infected intraperitoneally became positive at 9 days. An adult, infected intravenously, was positive in 13 days. Following these infections the "A" culture became avirulent and we were unable to obtain any infections subsequently. "B" culture maintained in the same manner as "A" has maintained its virulence. Observations on the prepatent period of "B" in both dogs and puppies seemed to indicate that subcutaneous inoculation produced a detectable infection a little sooner than the intraperitoneal route (Table 1).

In juveniles, infected subcutaneously, no significant differences were noted between the prepatent periods in the two sexes. The mean values were: 18 days (range: 13–32) for males (15 animals) and 19 days (range: 10–36) for females (21 animals). Although the range was greater in the females, represented by a

larger sample, than in the males, if the 21 values for females and the 15 values for males are divided into three groups, representing earlier, middle and later patencies it is found that the means for the earlier groups are very close (14.3 days for females; 14.4 for males) and the means for the later groups are identical (24 days for both sexes). The interval between the first positive and the last in any given litter varied from 1 to 12 days; the mean was 5 days.

In adults infected subcutaneously with "B", the prepatent periods in males (15, 20 and 20 days) were slightly shorter than in females (20, 21 and 22 days). In adults infected subcutaneously with "W", however, no consistency was observed. The periods in males were 7, 8 and 10 days; in females 5, 5 and 10. Com-

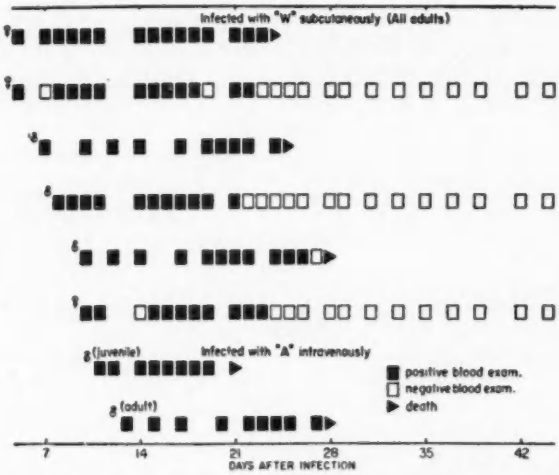

FIG. 1. Patent Periods of Experimental Chagas Disease in Dogs.

parisons between these unrelated animals of unknown ancestry and history may be unwarranted.

Patent Period. The periods of patency and times of death for all of the nonmedicated animals infected with "A" and "W" are shown in Figure 1. All of the dogs infected (subcutaneously) with "W" were adults, as was one of the animals infected (intravenously) with "A". It will be noted that 3 of the 6 adults infected with "W" showed spontaneous recovery. These animals were checked serologically for some time after the patent period and will be discussed later in relation to the agglutination tests.

Similar data for the nonmedicated adults which were infected with "B" are recorded in Figure 2. One of the females died in 32 days and all 3 of the males succumbed between 35 and 38 days after infection. Two of the females became patent but did not die; these were sacrificed 95 days after infection. Tissues taken at autopsy showed no evidence of the disease. One male, infected intravenously died in 40 days; one female, infected intraperitoneally, died in 37 days.

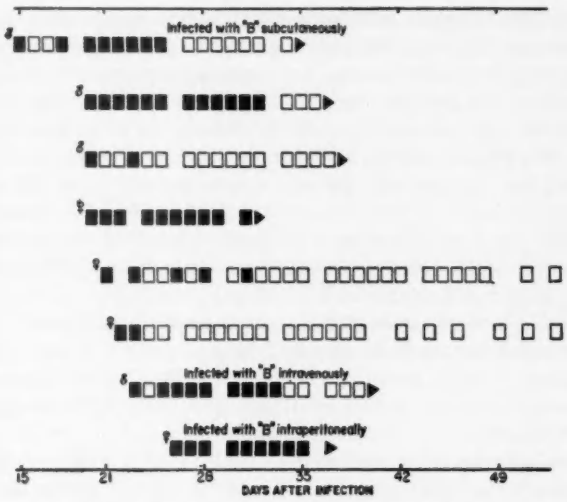

FIG. 2. Patent Periods of Experimental Chagas Disease in Dogs.

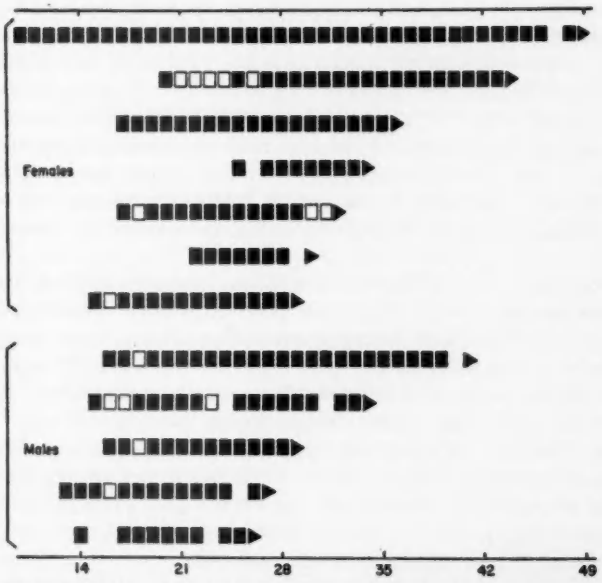

FIG. 3. Patent Periods of Experimental Chagas Disease in Juvenile Dogs—Infected with "B" subcutaneously.

In nonmedicated puppies infected subcutaneously with "B" (Fig. 3) there were no significant differences between the mean or median survival times of the two sexes, although the males tended to succumb a little sooner (mean, 31 days; median, 29) than the females (mean, 36 days; median, 34). The symptomatic aspects of the disease were not appreciably different in males and females. The parasitemias observed in males, however, were greatly in excess of those seen in females. On one test the peak parasitemias in 2 males were 120 and 165 organisms per high power field while the peak counts for 2 females of the same litter were 4 and 22. These ratios are not necessarily typical but consistently higher counts were obtained in males on all tests where identical conditions of infection and handling made comparisons on the basis of sex valid.

Parasitemias in juveniles were almost always greater than in adults; the highest counts recorded for the three strains in adults were: "A", one parasite per high power field; "B", 3 per h.p.f.; "W", 4 per h.p.f. Peak parasitemias in juveniles infected with "A" were lower than those with "B"; young dogs were not infected with "W".

High parasitemias were not consistently correlated with severe cardiac damage or short survival times. The animal which had the highest counts observed in a female (22 per h.p.f. at the peak) had the longest survival time of any nonmedicated female puppy (49 days). On the other hand several animals died from severe myocarditis after 8 to 20 days of patency without the parasitemic levels exceeding 10 organisms per 100 high power fields. Death rarely occurred at the peak of parasitemia; most commonly it ensued between 2 days and 2 weeks thereafter.

Paraplegic symptoms occurred in 4 dogs. Two of the animals (both females) had been infected with "B" and two (both males) with "W". One of the dogs with "B" (an adult) succumbed 28 days after a course of medication which completely cleared the parasitemia and produced an apparent clinical cure. The other (a juvenile) was killed 64 days after treatment, having recovered from paraplegia which appeared 20 days following medication and lasted about a week.

Both of the dogs with "W" were adults and had not been treated. One of these dogs became paraplegic at 26 days after infection and died 2 days later during the acute myocarditic phase of the disease. The other showed weakness and partial paralysis from the 33rd day after infection which was 12 days after the parasites disappeared from the blood. This animal was sacrificed one month later, its condition having remained static during this time.

Pathology. Although a detailed description of tissue changes is not intended for this report, certain generalities should be made concerning the histopathological findings. In all untreated cases death was attributable to acute, diffuse myocarditis and cardiac failure. Leishmania forms of *T. cruzi* were found in almost all of the visceral organs examined from animals either untreated or inadequately treated. Appreciable chagomata developed at the site of inoculation (subcutaneous) in only 2 of the dogs (with "W"). In several dogs leishmania forms were found in the brain without significant tissue reaction. In the four animals which developed paraplegia, however, distinct lesions were noted in the cerebrum and brain stem. Glial nodules and agranulocytic infiltrations were most common.

The tissue invasiveness of the three strains did not appear to differ appreciably. Since animals were not sacrificed during the acute stage of the disease, however, direct comparisons between tissues from animals infected for the same period of time could not be made. Inasmuch as the extent of tissue invasion is undoubtedly influenced by the duration of the infection as well as by age, sex and immunity, it was felt that significant quantitative data would not be furnished by parasite counts in tissues of animals which died at different times between 20 and 49 days.

Serology. The possibility of making a diagnosis by serological test before the parasites could be found in the blood was explored, in view of the desirability of early treatment in the acute myocarditis to which the puppies were highly susceptible. One of the more recent tests is that of Hauschka *et al.* (49) in which there is agglutination of living culture forms of *T. cruzi* when suspensions of the organism are mixed with dilutions of sera from experimental animals and naturally infected humans with Chagas' disease.

Although all of the sera which Hauschka tested were presumably from hosts in the postpatent or chronic phase of the infection, the possibility of detecting prepatent infections seemed worthy of trial. Accordingly, agglutination tests were run by Hauschka's method, on 8 untreated dogs (4 with "B", 4 with "W"), at intervals of a few days, from the time of infection until patency, then weekly thereafter, into the postpatent period.

Hauschka *et al.* (49) found that non-specific agglutination sometimes occurred with normal human sera at a 1:100 dilution even when "Culbertson" strain was used as antigen. During the present investigation it was found that non-specific agglutinations occurred with the sera from uninfected dogs, sometimes causing positive reactions at a 1:200 dilution. To avoid this confusing behavior, therefore, dog sera were tested at 1:1400 dilutions.

Positive reactions were obtained during the patent period with 3 out of 4 of the sera of dogs infected with "W" and 1 of 4 infected with "B". The reactions were clear cut at the 1:400 dilution and in Hauschka's notation would be read at 3 plus for the "W" sera and 2 plus for "B". Weakly positive reactions at 1:200, in the sera that were negative at 1:400, could not be distinguished from the non-specific agglutination which sometimes occurred with sera from normal uninfected dogs at that dilution.

The only dog which showed a positive reaction during the prepatent period was one which did not show either an early or a high parasitemia. The other dogs, all but one of which showed parasitemia before the above-mentioned, did not give positive serological reactions until sometime after parasites were detectable in the blood. It was apparent, therefore, that the appearance of agglutinating antibodies in the blood could not be regarded as a reliable harbinger of early subsequent parasitemia, and the state of the infection and relation to patency was unpredictable from the serological findings.

Only 2 sera, both from dogs with "W", were positive into the postpatent period. These dogs were sacrificed 6 weeks after the end of the patent period (9 weeks after infection) along with another animal, infected at the same time, the serum of which had never been positive. Leishmania forms were not found in the

tissues of the last mentioned dog, but in one of the animals whose serum was positive at the time of death a few foci of parasites were found in the myocardium. In the other dog no leishmania forms were found although foci of agranulocytic infiltration were found in the epicardium, myocardium and valves.

Apropos of a recent report (53) of a high heterophile agglutination titer in a patient with Chagas' disease, a heterophile antibody test was run on an infected untreated dog 19 days after infection (on the seventh day of parasitemia). No agglutination was observed with the serum from this animal nor with the serum from a normal uninfected dog. Sera from a normal human and a patient with infectious mononucleosis were run simultaneously and agglutinations were observed with both of these, to 1:14 and 1:448, respectively.

Chemotherapeutic Tests. Inasmuch as spontaneous recoveries occurred in adult dogs infected with *T. cruzi*, most of our chemotherapeutic testing was done in puppies, which invariably succumbed to the disease when untreated. In all cases to be reported here, the inoculum was "B" culture. Two puppies were infected intraperitoneally, 36 by subcutaneous injection.

For reasons which will be amplified in the discussion to follow it seemed desirable to test the chemotherapeutic activity of the active principle of Bayer 7602 (Ac), i.e. bis-(2-methyl-4-amino-6-quinolyl)diallylmalonamide, when administered intravenously. Preliminary tests on uninfected dogs had shown that 3.5 mgm. (base)/kgm. of this compound (one-half the intravenous LD_{50} for mice) could be administered intravenously to dogs once a day for 5 days without untoward effects. It was administered, therefore, to 4 infected puppies at 3.5 mgm./kgm./day, 2 receiving 7, 1 receiving 8, and 1 receiving 14 consecutive daily doses. A fifth animal also received 14 doses but 12 of these were given in 6 days, i.e. the dose was administered twice a day. No toxic effects were observed in any of the animals but 4 of the 5 succumbed to Chagas' disease in spite of treatment.

The 3 puppies which became patent between 20 and 27 days after infection had an average survival time of 31 days which was the same as that for the 2 controls which were also patent within the above period. The fourth puppy to succumb died 54 days after infection but this animal was not patent until 36 days. The puppy which survived was patent at 42 days and was medicated 7 times. This animal was sacrificed 2 months after the last medication, 111 days after infection. No leishmania forms of *T. cruzi* were found in the tissues taken at autopsy.

Pentaquine was used in the treatment of 6 puppies, which received total amounts from 90 to 410 mgm./kgm. *per os*. Previous testing of pentaquine in dogs had shown that oral doses of 15 mgm. (base)/kgm. were not tolerated; severe diarrhea and fatal intestinal intussusception ensued (1). In the present tests, therefore, no single dose greater than 10 mgm./kgm. was used. The usual signs and symptoms of 8-aminoquinoline toxicity were noted during medication in all of these animals, including paralysis of nictitating membrane, salivation, anorexia, impairment of pupillary reflexes, cyanosis and cachexia, appearing approximately in the order named.

Five of the 6 puppies treated with pentaquine survived the acute phase of Chagas' disease and 4 of the 5 showed no evidence of *T. cruzi* infection on autopsy at various intervals from 1 week to 2 months after the last disappearance of trypanosomes from the blood. In 4 of the 6 medicated cases the parasites disappeared from the blood during a preliminary course of treatment with from 60 to 120 mgm./kgm. but subsequently reappeared. In 3 of these 4 cases medication was resumed and from 45 to 320 mgm./kgm. additional drug was administered, cures being effected in each case. The other animal in which parasitemia recurred was not treated further and succumbed (Figure 4).

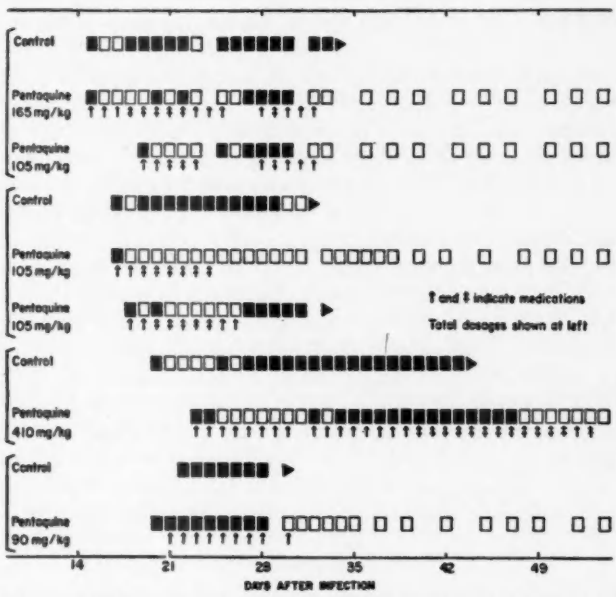

FIG. 4. Course of Experimental *T. cruzi* Infections in Dogs Treated with Pentaquine (Oral).

The puppy which received the lowest total dose (90 mgm./kgm.) did not show a parasitemic relapse following treatment but a few minute foci of leishmania forms were found in the myocardium 3 months later, although no cardiac damage was apparent and the animal was in perfect health at the time of its sacrifice. The puppy which was given the largest total dose (410 mgm./kgm.) was extremely tolerant and probably received considerably more drug than was necessary; 110 mgm./kgm. were given after the final disappearance of parasites from the blood. This puppy received 2 doses of 10 mgm./kgm. each on 11 consecutive days (20 mgm./kgm./day) without any more severe effects than those observed with half as much in the other animals.

Because of the successful use of as much as 20 mgm./kgm./day (in divided

doses) in the treatment of 1 puppy, this regimen was attempted in 1 adult dog which had been infected intravenously. This animal received 10 mgm./kgm. on the first day of patency, followed by 20 mgm./kgm./day for 2 days, and then 10 mgm./kgm. on the fifth day (a total of 60 mgm./kgm.). The organisms promptly disappeared from the blood but 4 days following the last dose the animal died with severe intestinal intussusceptions. No leishmania forms were found in the tissues. The puppy which tolerated the 20 mgm./kgm./day had previously been receiving 10 mgm./kgm./day for 16 days and had apparently become adapted to the continuous drug intake so that the higher dose was not fatal. The treatment of another adult dog (infected intraperitoneally) with a total of 60 mgm./kgm. (five days at 7.5, 15, 15, 15, and 7.5 mgm./kgm., respectively) prolonged its survival over the control but death ensued 68 days following infection after the appearance of paraplegic symptoms. Leishmania bodies were found in both heart and brain.

The minimum curative dose of pentaquine is probably about 100 mgm./kgm. and the resumption of medication when parasitemia recurs seems definitely indicated. Prolonged administration of amounts of drug which did not clear the organisms from the blood did not seem to result in the development of resistance, as evidenced by the ultimate cure in the animal which received the total of 410 mgm./kgm., 200 mgm./kgm. of which was given during an uninterrupted parasitemic period of 14 days, the longest ever observed in an animal which recovered.

In contrast with the successful treatments consummated with pentaquine, the establishment of a satisfactory therapeutic dosage regimen for isopentaquine was not accomplished. Only 1 puppy out of 4 treated was cured. The other animals could not be medicated often enough at high enough levels to get therapeutic effects. The toxic reactions at an initial dose of 5 mgm./kgm. were sometimes severe, although the puppy which was cured received 10 mgm./kgm./day for 8 consecutive days, after a preliminary course of treatment at 5 mgm./kgm./day. This animal is still alive and well, one year and a half after infection.

DISCUSSION

The greater susceptibility of young animals to experimental Chagas' disease has long been recognized. Likewise, the greater susceptibility of males is not surprising, in view of a number of reports on both protozoan and metazoan parasites. The sudden loss of virulence exhibited by "A" while being maintained in culture under conditions identical with those for "B", which continued to be avirulent, was an unanticipated development without apparent explanation. It is of interest that "W", which produces a distinctly virulent parasitemic infection in mice (47, 49, 54) does not produce infections either highly parasitemic or fatal in young rats (48) or uniformly pathogenic in dogs.

During the course of their studies on the neurotropic behavior of strains of *T. cruzi* isolated from armadillos, Villela and Magarinos Torres (23) noted that paralysis was usually observed in adult dogs, although they did record one case of paralysis in a puppy. They reported that in puppies the disease progressed more quickly to the acute parasitemic stage and there was not time for the appearance of nervous symptoms. Further, in arguing for the "special neurotropism of the

strain", they pointed out that dogs inoculated with *T. cruzi* from man did not become paralyzed.

Souza Campos' (20) record of experimental paralysis in dogs with a human strain, and the cases of paraplegia in the present study, indicate that neurotropism is not confined to armadillo strains. It seems to be clear, however, that paraplegic symptoms are typically observed in adult dogs which have been spared from the fatal myocardial form of the disease because of natural immunity or in young dogs which have likewise survived the acute infection because of chemotherapeutic treatment. The paucity of cardiac, in comparison with neural, lesions in the animals coming to autopsy at the paraplegic stage has been interpreted as confirmation of the neurotropic tendency.

In connection with the serological reactions certain reports of previous authors seem to require comment. In a critical review of some of the unsolved problems in chronic Chagas' disease, Yorke (55) called attention to the "difficult problem of ascertaining whether a positive (complement fixation) reaction implies actual infection or merely that the patient had once been infected." In the same year Johnson and Kelser (56) remarked, "If the positive complement fixation reactions in these cases can be taken as evidence that *T. cruzi* is still harbored somewhere in the body of the individual or lower animal, then a number of chronic cases or carriers have been encountered in this survey."

Johnson (39) described the histopathologic changes in 19 experimentally infected dogs, 2 of which died as a result of the infection, 6 of which were sacrificed during the patent period, 11 being allowed to live for various periods from 3 to 583 days after the disappearance of the parasites from the circulation. Parasites were found in the myocardia of the dogs which died, of those which were sacrificed during the patent period, and of the one sacrificed 3 days after patency, but in the animals which lived from 70 to 583 days thereafter no parasites were found, "even after long searches." With regard to "the probable presence of parasites in the tissues during the negative blood phase" Johnson says, "the fact that complement fixation tests are positive leaves little doubt as to their presence even though they cannot be demonstrated microscopically."

The failure to demonstrate parasitizing organisms in animals showing no evidence of the disease other than positive serological reactions is an embarrassing impasse of considerable importance for the interpretation of the results not only of immunologic tests but of chemotherapeutic experiments as well. If the examination of abundant histologic material cannot be used as a criterion of whether or not infection exists, the outlook with regard to eventual elucidation of the vagaries of chronic Chagas' disease must indeed be pessimistic.

The possibility that serologic tests for *T. cruzi* in dogs may give irregular results is suggested by observations of Minning (13) on the rhythmical variation of complement fixation reactions in experimental canine Chagas' disease as well as by those of Muniz and Freitas (57), who found that the correlation between agglutination and complement fixation reactions was good when human sera were tested but that armadillo and dog sera which were negative for agglutinins were positive in complement fixation tests.

Our interest in testing the intravenous efficacy of the active principle of Bayer

7602 (Ac) arose as a result of observations, both clinical and experimental, on the severe and lasting irritation produced by this compound on intramuscular administration. Although Fulton (58) pointed out, as a result of his tests in mice, that the compound was least effective when given intravenously, the recommendation of Mazza, Basso and Basso (59) that the injection of Bayer 7602 (Ac) be *"exclusivamente intramuscular"* seems to be attributable to Mazza's opinion that untoward effects resulted from intravenous injection, based on his observations in 2 patients who were given the product by that route (60). One of these patients was treated intravenously with 5 cc. of a 3% solution of Bayer 7602 (Ac) on each of 3 consecutive days. This was a total of 15 cc. containing 450 mgm. of the drug (360 mgm. of base) corresponding to about 5 mgm. (base)/kgm. (patient's weight was 71 kgm.). Abundant sweating was observed particularly on the head, lasting about one-half hour, accompanied by salivation and a bitter taste, as well as acceleration of the pulse and a feeling of cardiac oppression. The other patient received, intravenously, 3 cc. on one day and 5 cc. on the following day, or a total of 8 cc. containing 240 mgm. of drug (192 mgm. of base) or about 3.7 mgm. (base)/kgm. (weight was 52 kgm.). In this case there was also abundant sweating and the patient suffered from faintness during the half hour after injection. No indication of the rate of injection was given. In both of these cases salutory effects on the edematous manifestations of Chagas' disease were observed.

It was our desire not only to determine whether untoward reactions occurred in dogs as a result of intravenous injection of bis-(2-methyl-4-amino-6-quinolyl) diallylmalonamide but also to ascertain whether or not the course of the disease was altered by this type of medication. The failure to effect cures in 4 out of 5 animals so treated, one of which received a total of 49 mgm./kgm. of the compound, seems conclusive evidence for the rejection of this mode of administration. The animal which recovered was the one which had the longest prepatent period. Although our observations on the course of the disease in unmedicated puppies did not indicate a positive correlation between early patency and short survival, certain data of Villela and Torres (23) show this tendency. Five of their dogs with an average prepatent period of 9 days had an average survival time of 33 days, while another 5 with an average prepatent period of 20 days had an average survival time of 53 days. Exceptional cases, however, resembled ours, e.g., one animal (female) patent at 9 days lived 47 days, and another (male) patent at 24 days lived only 35 days after infection.

The successful use of pentaquine in the treatment of experimental Chagas' disease in puppies confirms previous observations on the chemotherapeutic activity of this compound in mice (1, 61). The suppression of symptoms, survival and absence of leishamania forms in the tissues at autopsy in the adequately treated cases are regarded as encouraging evidences of the curative value of pentaquine therapy.

When the comparative toxicities of pentaquine and iso pentaquine in animals and humans are studied a consistently contradictory pattern is apparent. The pamaquine equivalents in rats and monkeys as given by Schmidt (62) indicate

that isopentaquine is the more toxic: 0.40 in rats, 0.50 in monkey, in contrast to 0.30 and 0.25 for pentaquine. Our experiences with mice and dogs confirm this general pattern. In mice the relationship resembled that in monkeys, i.e. isopentaquine was twice as toxic as pentaquine, while in dogs our results resembled those of Moe, Peralta, and Seevers (63), suggesting that 7.5 mgm. of isopentaquine is the approximate equivalent in toxicity of 10 mgm. of pentaquine.

Alving's (64) observations in man, however, indicate a lesser toxicity for isopentaquine, the maximum tolerated dose being twice as great as that of pentaquine. Although this paradoxical relationship between toxicity in animals and man is not encouraging to the investigator who seeks a quantitative preclinical evaluation of tolerance, the outlook for the possible application of chemotherapeutically active 8-amino-quinolines in Chagas' disease has become more encouraging in the light of recent clinical research (65).

SUMMARY

1. Experimental Chagas' disease has been produced in adult dogs by inoculation with culture forms of "A" and "B" strains of *Trypanosoma cruzi* and with blood forms of "W" strain.

2. Occasional spontaneous recoveries occurred in adult dogs but untreated infections in puppies were uniformly fatal.

3. The prepatent periods for "W", and "A", and "B" strains were: 5 to 10 days, 9 to 13 days, and 10 to 42 days, respectively.

4. The prepatent period was a little shorter in animals infected subcutaneously than in those infected intraperitoneally.

5. The prepatent periods and the survival times did not differ between the sexes but the parasitemias in males were higher than in females.

6. In unmedicated puppies death from acute myocarditis ordinarily ensued between 8 and 39 days following the appearance of trypanosomes in the blood.

7. The median survival time in untreated infections in puppies was 34 days from the time of inoculation; the shortest was 27, the longest 49 days.

8. Paraplegia was occasionally observed in adult dogs infected with "B" and "W" and occurred once in a puppy which survived the acute myocarditic stage as a result of medication with Bayer 7602.

9. Agglutination tests, using live culture as antigen, did not give consistent results during any of the periods of the disease.

10. The intravenous administration of Bayer 7602 did not usually alter the course of Chagas' disease in puppies; isopentaquine was usually not curative in tolerated doses.

11. Pentaquine, given orally, produced cures in puppies as evidenced by suppression of symptoms, eradication of parasites from the blood, survival, and absence of leishmania forms in the tissues at autopsy.

12. Although isopentaquine is more toxic than pentaquine in animals, the opposite is apparently the case in man; either compound might prove to be useful in clinical Chagas' disease.

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SUSCEPTIBILITY OF CERTAIN SPECIES OF PANAMANIAN MONKEYS TO THE VIRUS OF ACUTE ANTERIOR POLIOMYELITIS

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During the recent epidemic of acute anterior poliomyelitis in Panamá the author became interested in experimental studies of the etiologic agent. Three strains of poliomyelitis virus were isolated in rhesus monkeys (Rodaniche, in press). However, in view of the costliness of *Macaca mulatta* and the delay and difficulty experienced at times in obtaining specimens, it seemed advisable to attempt transmission of the virus to local monkeys. Since Melnick and Paul (1943) and Melnick (1946) had reported successful transmission to South American species of *Cebus capucina*, the corresponding Panamanian species was chosen for study. Later, experiments were also conducted with two of the four local subspecies of the howler monkey and with juvenile specimens of the black spider monkey. Mackay and Schroeder (1935) were unsuccessful in their attempts to transmit the virus to the South American spider monkey, *Ateles ater*, but we hoped that perhaps infant and juvenile specimens might prove more susceptible.

The species of Panamanian monkeys utilized in these studies according to the latest classification by the Smithsonian Institution were:

Whiteface monkey—*Cebus capucinus imitator*

Howler monkey—*Alouatta palliata palliata*

Alouatta palliata aequatorialis

Black spider monkey—*Ateles fusiceps robustus*

Whiteface monkeys were available in fair numbers, as this animal has a wide sale as a pet and more intensive efforts are made, therefore, to capture it. Unfortunately, many specimens are maintained alive in the laboratory only with difficulty due to the heavy infestations with parasites to which they are prone. Relatively few howler monkeys were available. Although this is one of the most abundant species in Panamá, little effort is made to capture it alive as there is no market for it as a pet. Due to dietary idiosyncracies the howler is troublesome to maintain in the laboratory for more than a few weeks. However, if patience is exercised in training it to a laboratory diet, it thrives well.

Virus was successfully transmitted to *Cebus capucinus imitator*, *A. palliata palliata* and *A. palliata aequatorialis*. To the best of our knowledge this is the first report of successful transmission to the latter species. Only two *Ateles fusiceps robustus* were inoculated. As these gave negative results, and in view of the previous report of Mackay and Schroeder (1935), investigation of these animals was abandoned.

VIRUS STRAIN

The virus utilized in the experiments here reported was a strictly monkey-pathogenic strain (non-transmissible to rodents), obtained from the spinal

cord of a 2 year old male infant (A.A.) who died of clinically typical poliomyelitis, confirmed by post-mortem pathological studies, in the Santo Tomas Hospital, Panamá, R. P. in August of 1950 at the beginning of the outbreak. Primary isolation was made in *M. mulatta*.

METHOD

Inoculation was made in most instances by the combined intracerebral, intranasal and intraperitoneal routes as Melnick and Paul (1943) reported greater success by this method. Intracerebral dosage was graduated from 0.5 to 1 cc according to the weight of the monkey and injection was always unilateral. Ten per cent centrifuged suspensions of cord tissue in physiologic saline solution were employed. Temperatures were taken rectally once daily at approximately 10 A.M. The majority of animals were sacrificed soon after the onset of paralyses. The usual criteria for pathological diagnosis were employed: focal, diffuse and perivascular round cell infiltration most prominent in the anterior horns of the spinal cord, neuronal necrosis and neuronophagia. After each successive monkey passage, 6 adult and 6 infant white mice, 2 guinea pigs, 2 hamsters and one rabbit were inoculated intracerebrally with consistently negative results.

The rhesus monkeys were healthy young tuberculin-negative specimens weighing from 2500 to 3200 grams. Six of the 8 cebus monkeys were young adults or juveniles weighing from 900 to 2200 grams. Two infant cebus monkeys #391 and #413 weighing 690 and 525 grams respectively were used. With one exception all howlers were juvenile or infant specimens weighing from 1050 to 1595 grams. Howler #462 was a young adult weighing 4380 grams. The two black spiders were one infant and one juvenile specimen weighing 1375 and 2510 grams respectively.

RESULTS

A fairly detailed tabulation of results obtained is presented in Table I.

Susceptibility of Cebus capucinus imitator

This species was found to be quite susceptible to the A.A. monkey-pathogenic strain of poliomyelitis but only after various passages of this strain in the rhesus. One cebus inoculated with human cord, two with first passage rhesus cord and one with second-passage rhesus cord failed to develop symptoms or pathological changes suggestive of poliomyelitis. Infection was obtained for the first time with third rhesus passage cord in both whitefaces injected. After the third passage in rhesus monkeys the virus was passed twice in succession in howler monkeys, and one cebus was inoculated with howler cord after each passage. Positive results were obtained in both cebus monkeys thus inoculated with howler cord. Symptoms in cebus monkeys were less severe than in either *M. mulatta* or *Alouatta palliata*. After an incubation period of 4 to 5 days febrile temperatures were noted in 3 of the 4 monkeys giving a positive result, followed in 2 days by the onset of paralyses which affected one or both posterior ex-

TABLE I

Susceptibility of Various Species of Panamanian Monkeys to the A,A. Strain of Acute Anterior Poliomyelitis

INOCULUM UTILIZED	NO. OF PREVIOUS MONKEY PASSAGES*	NO. DAYS CORD WAS PRESERVED IN GLYCERINE PRIOR TO FURTHER PASSAGE	SPECIES AND NO. OF MONKEYS INOCULATED.	1ST DAY OF FEVER	1ST DAY OF PARALYSIS	EXTENT OF PARALYSIS	FATE OF MONKEY	PATHOLOGICAL CHANGES IN SPINAL CORD
Human cord (AA)	0	3	Rhesus #334	6	9	2H	S9	Pos.
			Whiteface #391	—	—	—	D47	Neg.
Human cord (AA)	0	264	Rhesus #426	10	14	1H	Alive	—
			Howler #486	—	—	—	Retested†	
			Howler #487	—	—	—	D27	Neg.
Cord of Rhesus #334	1	2	Rhesus #333	10	11	2H & 1F	S14	Pos.
			Whiteface #394	—	—	—	Alive	—
			Whiteface #413	—	—	—	D38	Neg.
Cord of Rhesus #333	2	181	Rhesus #425	4	6	2H	S8	Pos.
Cord of Rhesus #333	2	285	Whiteface #455	—	—	—	D19	Neg.
			Howler #492	—	15	2H & 2F	S16	Pos.
Cord of Rhesus #425	3	6	Whiteface #452	5	7	2H	S8	Pos.
			Whiteface #453	—	8	1H	Alive	—
			Howler #462	5	8	2H & 2F	S8	Pos.
			Spider #442	—	—	—	Alive	—
			Spider #454	—	—	—	Alive	—
Cord of Howler #462	4	26	Whiteface #457	4	6	1H	D48	NT
			Howler #482	4	6	2H & 2F	S6	Pos.
			Rhesus #429	4	7	2H	Alive	—
Cord of Howler #482	5	51	Whiteface #480	5	7	2H	Alive	—
			Howler #491	7	11	2H & 2F	D17	Pos.
			Howler #486	6	8	2H & 2F	S8	Pos.

* The first three passages were effected in rhesus monkeys. Passages no. 4 and 5 were made in howler monkeys.

† Reinoculated 46 days after first inoculation.

H indicates hindleg. F indicates foreleg. S9 indicates sacrifice of animal in 9 days. D47 indicates death of animal in 47 days.

tremities. No paralyses of the upper extremities were observed. Melnick and Paul (1943) suggest that positive results in cebus monkeys may be favorably influenced by the proximity of the virus to its human origin. Our results with the A.A. strain isolated from human cord indicate that previous passages in the rhesus monkey favored infection of the cebus monkey.

Susceptibility of Alouatta palliata

Howler monkeys became available for this study only after three passages of the A.A. human cord strain had been effected in *M. mulatta*. A single howler #462 (see Table I) was inoculated with this third passage rhesus cord virus with a successful result. In order to test the susceptibility of this species to virus in human cord, we were obliged to resort to inoculation with the original human cord (A.A.) after more than 8 months of preservation in glycerine solution. One *M. mulatta* and two juvenile howlers were injected. One of the howlers died of other causes in 27 days. Its cord was negative for characteristic pathological changes. The other howler remained alive and well and was reinoculated 46 days later with a suspension of the cord of howler #482 (see Table I). It developed a rapid and severe infection indicating absence of immunity. The rhesus, on the other hand, developed a mild infection with fever and paralysis of one hindleg ten days after inoculation.

Due to scarcity of animals no attempt was made to pass first generation rhesus cord virus to the howler. However, second rhesus passage virus preserved 285 days in glycerine solution gave a positive result in the one howler injected, #492, characterized by a quadriplegia which developed 15 days after inoculation. A cebus simultaneously inoculated gave a negative result.

Three howler monkeys proved fully susceptible when inoculated with virus in homologous cord tissue in the 4th and 5th monkey-passages, the first three of which were effected in the rhesus and the last two in the howler monkey. Two cebus and one rhesus monkey were similarly successfully infected with howler cord virus. It is to be noted that all of five howler monkeys inoculated with second to fifth monkey passage virus developed the paralytic disease, including 4 specimens identified as *Alouatta palliata palliata* and one, #492, identified as *A. palliata aequatorialis*. The incubation period varied from 4 to 11 days followed by one or more days of fever. One animal presented paralyses on the 15th day without a previous rise in temperature having been observed. In two howlers light tremors of the head and incessant crying were noted at the beginning of the febrile period. In all 5 animals there was almost simultaneous involvement of all four extremities. Characteristically, the monkey would be noted to show apathy and inappetence one day and the next day would be found lying prostrate on the floor of the cage. The hindlimbs usually showed more extensive paralysis than the forelimbs. Four of the five animals were sacrificed within one to two days of the onset of paralysis. The fifth #491 became progressively weaker, showed gasping difficult respiration and died 17 days after inoculation, 6 days after the onset of paralysis.

Inability to infect Ateles fusiceps robustus

Only two black spider monkeys were used in the present experiments, one juvenile and one infant. These were inoculated with third rhesus passage cord with a completely negative result. As two whiteface and one howler monkey similarly inoculated all developed symptoms, it seemed of little value to pursue attempts to infect this species.

DISCUSSION

The results here reported indicate that the Panamanian whitfeace monkey, *Cebus capucinus imitator*, and at least two subspecies of the howler monkey, *Alouatta palliata*, are susceptible to infection with the virus of acute anterior poliomyelitis. However, the howler would appear to be preferable for experimental work since it showed a more uniform susceptibility and developed a much severer and more easily recognizable infection. If we can judge by a single experiment using one rhesus monkey, two howlers and one virus strain, the howler would seem to be less suitable than *M. mulatta* for use in primary isolation of virus from human nervous tissue. No tests were made of the susceptibility of these species to single routes of injection or to virus in stool suspensions due to the scarcity of animals. We hope to be able to test this phase of the experimental disease in the future, if we can obtain an adequate supply of monkeys and if there are sufficient human cases available. In the experiments reported here, age apparently did not influence susceptibility of the howler to any important degree as one adult, three juvenile and one infant animals all proved equally susceptible.

CONCLUSIONS

1. Monkey-pathogenic poliomyelitis virus proved infective to two species of Panamanian monkeys, *Cebus capucinus imitator*, the whiteface monkey, and *Alouatta palliata*, the howler monkey. This is the first report of successful transmission to the latter genus.

2. Howler monkeys were infected with second and third rhesus passage cord and with howler cord suspensions in the 4th and 5th monkey passages. Virus in howler cord could also be transmitted to *M. mulatta* and *C. capucinus*.

3. In a single experiment we failed to infect *A. palliata* with human cord virus which had become somewhat reduced in virulence after 8 months preservation in glycerine solution.

4. Infection in the howler monkey was invariably severe with rapid development of quadriplegia after a variable incubation period.

5. The uniform susceptibility of *Alouatta palliata* to monkey passage virus and the marked clinical manifestations elicited by infection in this animal should make this species of value in experimental studies.

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PERIODICITY IN THE ANNUAL INCIDENCE OF REPORTED CASES OF YELLOW FEVER DURING THE PAST FIFTY YEARS[1]

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Many infectious diseases, some caused by bacteria and others by viruses, show a marked periodicity. This type of epidemiological pattern occurs alike, whether the pathogens are water-borne, air-borne, transmitted by contact or carried by blood-sucking insects. Some of these illnesses exhibit fairly regular cyclical fluctuations in prevalence, while others behave in a completely erratic manner.

It was noticed more than 50 years ago by those who had made careful studies of such maladies that outbreaks of unusual severity did not occur annually, but tended rather to make their appearance after quiescent intervals lasting several years. Hirsch (1883) stated that during the latter part of the nineteenth century four pandemics of yellow fever broke out on the South American continent, separated by intervals of four, six and 11 years respectively. Similarly each of the major outbreaks of cholera that occurred in India during the past century was followed by periods of low prevalence of several years' duration.

Hedrich (1933) made a careful analysis of case records of measles in Baltimore, Maryland, from 1899 to 1931 inclusive. He found that epidemics of that illness appeared every two or three years, though occasionally smaller epidemics were registered annually for several successive seasons. Realizing only too clearly the inaccuracies of his basic data, Hedrich made appropriate corrections wherever he could. In England Hamer (1906) and Greenwood (1935) observed that outbreaks of measles had a periodicity of roughly 18 months or two years, though an adequate explanation of why the incidence of this disease increased every two or three years has not yet been found.

Brownlee (1919) studied the sequence of epidemics of influenza in England between 1889 and 1917. He concluded that periods of unusual prevalence appeared at intervals of 33 weeks except when such a period fell due in the autumn months. After an investigation of 16 widespread epidemics of influenza, which occurred in the United States between 1920 and 1944, the Commission on Acute Respiratory Diseases (1946) reported that influenza "A" appeared to have a cycle of two to three years and influenza "B" one of four to six years. Events which have occurred since 1946, however, have failed to support the hypothesis of such a regular periodicity of influenza.

Another disease in which periodicity has been a striking characteristic is epidemic cerebrospinal meningitis. Gover and Jackson (1946) reported unusual prevalence of this illness in the United States in 1905, 1918, 1929, 1936 and 1943. The median interval was nine years and the extremes seven and 13 years. In the

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Division of Medicine and Public Health of The Rockefeller Foundation.

nineteenth century the magnitude of epidemic waves of meningitis varied widely. However, a year of unusual incidence could be expected at least once in each decade.

Chickenpox, scarlet fever, mumps and diphtheria show a similar tendency to periodicity, particularly when they are observed in a single locality. During the period from 1915 to 1949 New Zealand (Maclean, 1950) experienced four major epidemics of poliomyelitis separated by intervals of 9, 12 and 11 years. These outbreaks occurred in 1916, 1925, 1937 and 1948. Prior to 1916 only sporadic cases had been recorded in that country.

This phenomenon has been observed also in the most important of the insect-borne diseases, namely, malaria. In a famous study carried out by Christophers (1911) comparative data were assembled for food prices, rainfall and malaria in Punjab, India, from 1869 to 1908. Six very severe epidemics of malaria were registered during those four decades and these occurred at intervals of from five to nine years with a median period of eight years. Peak food prices resulting in widespread famine accompanied the peaks of malaria fatalities. Interestingly enough rainfall showed a poorer degree of correlation with excessive malaria incidence than did food prices.

Gill (1928) pointed out that while major epidemics of malaria have exhibited no constant periodicity, at Amritsar in central Punjab, India, a lapse of at least five years always intervened between severe recurrences of this disease. He applied the term "secular periodicity" to this phenomenon. In Algiers, North Africa, Sergent (1932) observed three major epidemics between 1902 and 1930. They occurred in years of excessive rainfall and after intervals of 13 and 11 years respectively. Gabaldón (1949) spoke of the "paraquinquennial periodicity" of malaria in Venezuela. In the State of Carabobo peaks of unusual malarial incidence occurred in 1915, 1920, 1926, 1931 and 1942. In that area, therefore, the intervals varied from five to 11 years with a median value of five and a half years.

THE PERIODICITY OF YELLOW FEVER

Apart from the pandemics of the nineteenth century, which were described by Hirsch (1883), no recent inquiry into the periodicity of yellow fever in South America has been carried out. The extensive epidemics of sylvan yellow fever, which spread over the southern portion of the State of Goias and contiguous areas in the State of Minas Gerais, Brazil did, however, call attention to such a possibility. Maps 1 and 2, which were furnished through the kindness of Dr. Waldemar da Silva Sa Antunes, Director of the National Yellow Fever Service of Brazil, indicate a striking similarity between the regions attacked during 1935 and those invaded 10 years later. It is also noteworthy that an outbreak of jungle yellow fever occurred on the "Planalto" of Mato Grosso in 1934 and that this was followed in 1944 by several additional fatal cases. One of the individuals who succumbed to the disease was infected in the same forest in which a relative of his had contracted the disease 10 years previously.

In order to find out whether this apparent 10-year periodicity was really of

TABLE 1

Annual prevalence of reported cases of yellow fever from 1901 to 1950 inclusive. (Public Health Reports, U. S. Marine Hospital Service and U. S. Public Health Service)

YEAR	TOTAL FOR AFRICA	BOLIVIA	BRAZIL	CARIBBEAN ISLANDS	CENTRAL AMERICA AND PANAMA	COLOMBIA	ECUADOR	MEXICO	PERU	VENEZUELA	U. S.	ALL OTHER AMERICAN COUNTRIES	TOTAL FOR THE AMERICAS
1901	—	—	331	*203*	47	6	—	260	—	—	—	—	847
1902	—	—	479	13	147	—	19	843	—	1	—	67	1569
1903	—	—	*781*	14	224	4	*248*	*4612*	—	6	—	—	5889
1904	—	—	254	16	41	4	133	437	5	7	7	—	904
1905	2	—	*833*	86	*2035*	11	132	212	18	13	*8407*	59	*11806*
1906	35	—	162	109	61	—	*258*	170	9	—	7	—	776
1907	—	—	405	*213*	9	1	131	8	2	*294*	6	—	1069
1908	—	—	388	165	3	—	117	128	—	81	2	18	902
1909	—	—	499	99	1	1	272	52	1	11	—	15	951
1910	16	—	*750*	3	8	—	286	11	—	55	—	—	1113
1911	9	—	274	1	—	1	306	44	*76*	89	—	—	791
1912	7	—	151	4	1	1	*363*	*164*	43	*91*	—	*1134*	*1952*
1913	36	—	117	15	—	1	340	34	—	6	—	—	513
1914	4	—	83	17	—	—	39	8	—	8	—	—	155
1915	2	—	4	—	—	4	20	4	—	—	—	—	32
1916	—	—	—	21	—	—	281	34	—	—	—	—	336
1917	—	—	19	—	1	—	103	25	—	11	—	—	159
1918	—	—	36	—	*610*	—	*434*	2	—	—	—	—	*1082*
1919	—	—	*108*	—	19	—	145	47	173	—	—	—	492
1920	—	—	7	—	71	1	—	*301*	*455*	—	—	—	835
1921	—	—	1	5	20	—	—	120	294	—	—	—	440
1922	—	—	7	1	—	—	—	20	—	—	—	—	28
1923	—	—	*154*	—	—	*39*	—	1	—	—	—	—	194
1924	8	—	2	—	80	—	—	—	—	—	—	—	82
1925	38	—	—	—	—	—	—	—	—	—	—	—	—
1926	43	—	41	—	—	—	—	—	—	—	—	—	41
1927	346	—	—	—	—	—	—	—	—	—	—	—	—
1928	62	—	143	—	—	—	—	—	—	—	—	—	143
1929	33	—	*672*	—	—	*57*	—	—	—	—	—	—	*729*
1930	4	—	6	—	—	—	—	—	—	—	—	—	6
1931	58	—	46	—	—	4	—	—	—	—	—	—	50
1932	38	30	32	—	—	—	—	—	—	—	—	—	62
1933	80	—	14	—	—	—	—	—	—	—	—	—	14
1934	59	—	14	—	—	3	—	—	—	—	—	—	17
1935	30	1	110	—	—	17	—	—	—	—	—	—	128
1936	43	16	177	—	—	*66*	—	—	—	—	—	—	259
1937	171	—	185	—	—	53	—	—	23	—	—	1	262
1938	76	—	*274*	—	—	15	—	—	—	—	—	—	*289*
1939	58	—	125	—	—	10	—	—	—	—	—	—	135
1940	864	1	151	—	—	35	—	—	—	—	—	—	187

TABLE 1—*Continued*

YEAR	TOTAL FOR AFRICA	BOLIVIA	BRAZIL	CARIBBEAN ISLANDS	CENTRAL AMERICA AND PANAMA	COLOMBIA	ECUADOR	MEXICO	PERU	VENEZUELA	U. S.	ALL OTHER AMERICAN COUNTRIES	TOTAL FOR THE AMERICAS
1941	36	—	16	—	—	*47*	—	—	5	1	—	—	69
1942	17	26	6	—	—	18	—	—	—	2	—	—	52
1943	32	—	2	—	—	31	—	—	—	—	—	—	33
1944	13	4	15	—	—	14	—	—	—	12	—	—	45
1945	18	*42*	*102*	—	—	23	—	—	7	33	—	1	*208*
1946	51	40	1	—	—	21	—	—	3	12	—	—	77
1947	4	5	2	—	—	*66*	—	—	3	—	—	—	76
1948	5	—	3	—	—	19	—	—	2	1	—	2	27
1949	33	—	5	—	11	—	1	—	4	—	—	—	21
1950	22	*867*	22	—	1	10	—	—	14	3	—	—	*917*
Total	2353	1032	8009	985	3390	583	3628	7537	1137	737	8429	1297	36764

frequent occurrence or merely an exceptional situation, it was decided to make a careful inquiry into the past history of yellow fever, at least since 1900.

During the first half ot the twentieth century records of the prevalence of reported cases of yellow fever have been published by the United States Public Health Service. This information was originally furnished in the form of consular reports, which were sent in from various countries affected by this disease. The consular reports from the American republics have been more complete than those furnished by various colonies and protectorates on the African continent.

Table 1 indicates the distribution by years and countries of 36,764 cases of yellow fever which were reported from the Americas, and of 2,353 cases diagnosed in Africa. The fact that 94 per cent of these cases were recognized in the Western hemisphere does not necessarily indicate that this disease is more common in the New World. In all probability this difference merely shows that the reporting of cases has been more complete in the Americas. This difference was particularly noticeable prior to 1925. Because the number of cases reported from Africa is relatively small, the cases have not been subdivided according to countries.

The numbers of cases in years of unusual severity have been underlined. This device shows that in Brazil there have been eight peaks of high incidence during the past 50 years and five in Colombia. Yellow fever became unusually prevalent in Ecuador on four occasions, before the urban form of the disease was eradicated from Guayaquil in 1919. In the city of Guayaquil itself there were five peak years of reported cases, namely 1903, 1906, 1910, 1916 and 1918. Similarly, three major outbreaks were recognized in Mexico prior to 1923, the epidemic of 1903 having been particularly violent.

The extensive epidemic of 1905 in Central America involved the republics of Guatemala, Honduras and Panama. Then a period of 13 years elapsed before the

next severe outbreak in Guatemala (1918), in which there were more than 600 reported cases. Since 1924 the urban form of yellow fever has not reappeared in Central America.

It is surely significant that the years of unusual prevalence are not the same in all of the countries involved. The year 1940, for instance, saw the first epidemic in the Anglo-Egyptian Sudan from which 858 cases were reported, while elsewhere the disease was at a low ebb. Similarly, 1950 was the occasion of the largest known outbreak in Bolivia, while in other countries very few cases were encountered.

The period of time which has elapsed between successive peaks of high prevalence of yellow fever has varied from two to 13 years with a mean interval of 6.4 years and a median of six years. This data is summarized in Table 2.

TABLE 2

Intervals of time between successive peaks of unusual prevalence of yellow fever

ELAPSED TIME IN YEARS	NUMBER OF OBSERVED INSTANCES
2	1
3	1
4	1
5	4
6	6
7	2
8	1
9	4
10	—
11	—
12	—
13	1

Being fully aware of the inaccuracies in the current methods of reporting cases as well as the persistence of the disease in many so-called "silent yellow fever foci," Soper, Rickard and Crawford (1934) introduced a system for the routine postmortem removal of liver tissue from rapidly fatal cases of fever. Partial autopsies were carried out during 1930 in the States of Para, Rio Grande do Norte and Rio de Janeiro, Brazil. In February 1931 viscerotomes were distributed to selected representatives in the interior of the State of Pernambuco.

The growth of the viscerotome service was very rapid. From 1,500 liver specimens secured in 1931 and 13,733 obtained in 1932, this program was expanded by 1940 to more than 30,000 viscerotomies per year in Brazil alone. By 1949 more than 400,000 liver samples had been collected and examined. This viscerotome service was soon extended to Bolivia and Colombia.

Liver specimens were obtained with a viscerotome from cadavers of persons who had died of acute illnesses of not more than 10 days' duration. Cases of death from accident, homicide, puerperal sepsis or death of infants less than one month of age were excluded. From March 1933 on, no infants less than six months of age were punctured.

Table 3 gives the number of fatal cases of yellow fever diagnosed pathologically or else by the isolation of virus in Bolivia, Brazil and Colombia between the years 1930 and 1950. For the sake of completeness, 389 deaths recorded in Brazil in the year 1929 have been included, but as these were not all confirmed pathologically they are shown in parentheses only. Naturally, the figures presented

TABLE 3

Cases of yellow fever diagnosed pathologically or else by isolation of virus, which have occurred in Bolivia, Brazil and Colombia between 1930 and 1950

YEAR	BOLIVIA	COLOMBIA	URBAN	BRAZIL SYLVAN	SUB-TOTAL	TOTAL
(1929)*	—	—	(389)	—	*(389)**	—
1930	—	—	84	—	84	84
1931	—	—	125	—	125	125
1932	13	—	67	4	71	84
1933	1	—	18	1	19	20
1934	—	12	8	6	14	26
1935	4	25	18	112	130	159
1936	*56*	*73*	6	166	172	*301*
1937	2	59	3	214	217	278
1938	—	15	—	*262*	*262*	277
1939	5	10	—	132	132	147
1940	5	22	—	180	180	207
1941	2	*54*	—	19	19	75
1942	*36*	19	3	7	10	65
1943	—	38	—	6	6	44
1944	1	20	—	15	15	36
1945	8	25	—	*112*	*112*	*145*
1946	19	19	—	1	1	39
1947	5	77	—	2	2	84
1948	7	12	—	3	3	22
1949	5	3	—	6	6	14
1950	*27*	12	—	4	4	43
Total	196	495			1584	2275

* Many of the deaths from yellow fever, which were reported during the year 1929, were not confirmed pathologically.

in Table 3[2] are much more reliable than those given in Table 1, but nevertheless both tables show a similar pattern. The intervals of time between years of unusual prevalence of fatal cases of yellow fever, as shown in Table 3, varied from

[2] It is a pleasure to acknowledge the help received from colleagues in South America in supplying data for Table 3. Dr. George Bevier provided the information about Bolivia, Dr. Augusto Gast Galvis sent data from Colombia, and Dr. Waldemar da Silva Sa Antunes supplied the figures for Brazil from 1929 to 1950 inclusive.

five to nine with a median value of six and a half years. It should be emphasized also that, with the exception of very few cases diagnosed by the isolation of yellow fever virus, all the figures in Table 3 refer to deaths while those in Table 1 include recovered as well as fatal cases.

Jungle yellow fever was discovered in 1932 in the Valle do Canaan, Brazil. Most of the deaths from that disease, which have occurred in Brazil since 1935, have been ascribed to the jungle type. Table 3 shows that among 130 fatalities diagnosed during that year only 18 were urban in origin while the other 112 persons had been infected in the forests. Except for three cases of *Aedes aegypti*-transmitted yellow fever, which were observed at Sena Madureira in the Acre Territory in 1942, no more urban cases of yellow fever have been encountered in Brazil since 1937.

All of the yellow fever cases reported from Colombia since 1934 have been jungle yellow fever. A small urban outbreak occurred in Bolivia in 1932, but after 1936 all outbreaks in that country have also been of the sylvan type. It appears from a comparison of Tables 1 and 3 that a similar periodicity occurs in the two forms of the disease.

An area in Colombia, which has been under continuous observation since 1934, is the Intendencia of Meta. This region includes the towns of Acacias, Restrepo and Villavicencio. There have been three years of unusual incidence of yellow fever in the Intendencia of Meta, namely, 1936, 1941 and 1947. Those years correspond excatly to the peak years for the entire country.

In the Department of Santa Cruz, Bolivia, unusually large numbers of diagnosed yellow fever cases were recorded in the years 1932, 1936, 1942 and 1946. The periodicity in that area therefore varied from four to six years. The year 1950 showed a peak for Bolivia as a whole, but this was mainly due to an explosive outburst that took place in a region far to the south of Santa Cruz.

The accompanying graph shows the peaks of incidence of definitely diagnosed cases of yellow fever that have occurred in three of the South American republics during the past 20 years. It is again obvious that the years of unusual prevalence have not been the same in all of those three countries. For instance, 1936 was a peak year in Bolivia and Colombia, but not in Brazil. Similarly, a severe outbreak occurred in Brazil in 1945, which was followed two years later by an unusual increase of fatal cases in Colombia. The most recent major epidemic in Bolivia took place in 1950.

SUMMARY

1. An analysis has been made of the periodicity of severe epidemics of yellow fever in Africa and South America during the past 50 years.

2. Successive peaks of unusual prevalence of reported cases of that disease have recurred after intervals of from two to 13 years. No consistent pattern has been discernable although the most frequent interval was six years.

3. The periodicity of large numbers of fatalities, as revealed by the viscerotome service, has likewise been irregular and has shown a median value of six and a half years.

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(CHARTS I AND II ON FOLLOWING PAGES)

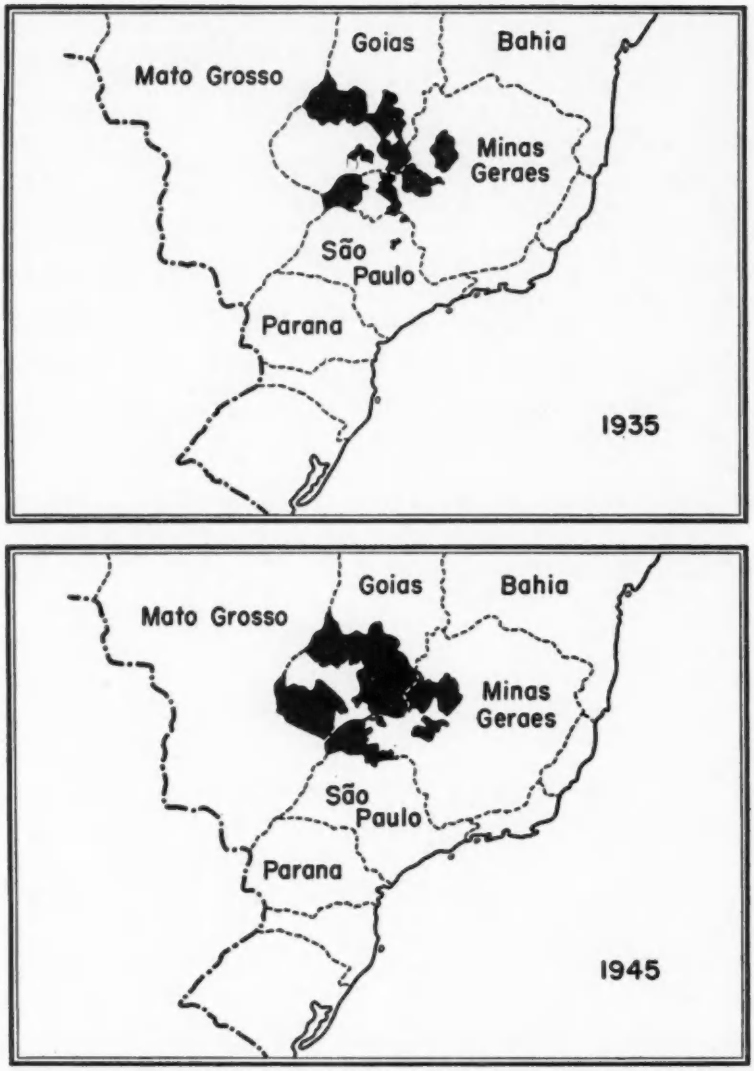

CHART I. Total area of "municipios" in Brazil in which cases of yellow fever occurred during the epidemics of 1935 and 1945.

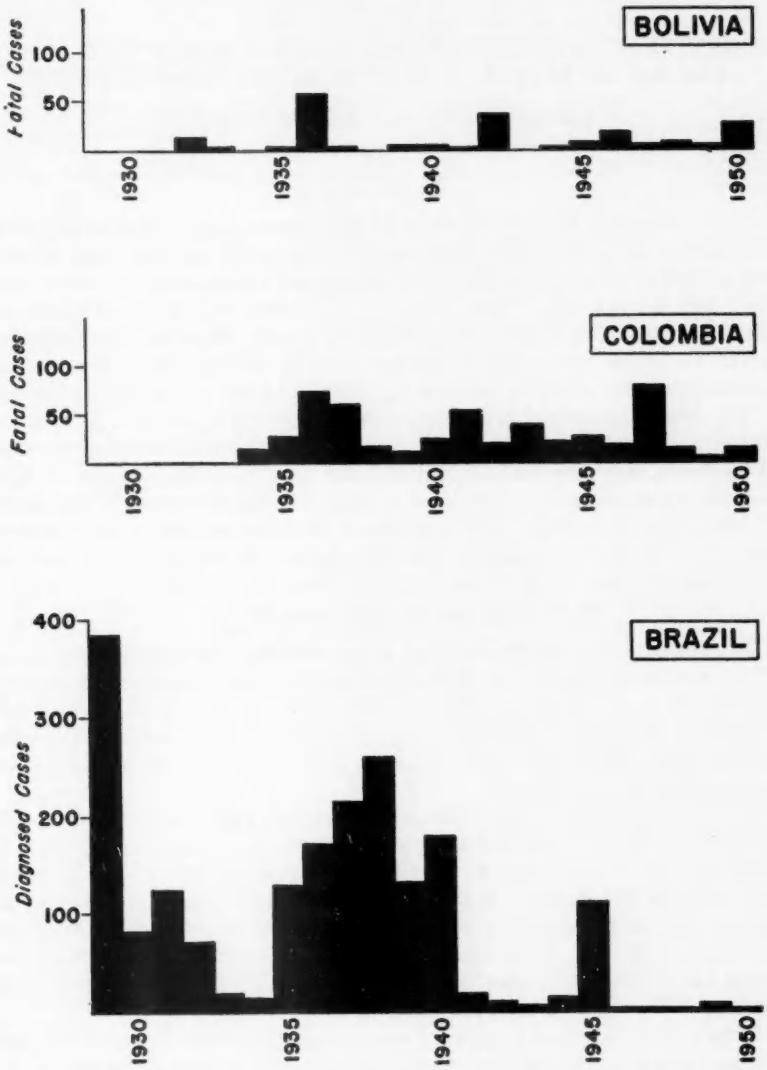

CHART II. Cases of yellow fever diagnosed pathologically or by isolation of virus.

QUANTITATIVE STUDY OF THE AMINO ACIDS OF THE BLOOD PLASMA OF AFRICAN PATIENTS AFFLICTED WITH YAWS

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INTRODUCTION

There is among the patients afflicted with yaws a high incidence of osteitis and periostitis involving the small bones of the hands and feet, long bones of the upper and lower extremities, nasal bones, and occasionally, the bones of the skull and thoracic cage. There is also often formation of nodules of fibrous tissue in the vicinity of appendicular joints, ulcerations and gummatous formation. Because of these pathological manifestations, it was thought that certain abnormalities of the nitrogen metabolism might be caused by *Treponema pertenue*.

The present investigation was conducted to check the possibility of an abnormal nitrogen metabolism related to yaws. The investigation included the study of the total nonprotein nitrogen (N.P.N.), the total free amino nitrogen (NH_2)N, and the determination of individual amino acid concentrations of the plasma of African patients with yaws. As several of the patients were under treatment, the effects of the treatment on the nitrogenous components studied are also reported.

MATERIALS AND METHODS

The patients on whom the analyses were conducted were hospitalized but not all were bed patients. Due to their longer period of hospitalization, more analyses were made on certain patients. A total of eight yaws cases was investigated. A summary of their condition is given in Table 1. The table includes brief information on the stage and severity of yaws, age of the patient, serology, complications, and type of treatment.

As the condition of all the patients was complicated by ascariasis or ancylostomiasis, it was necessary to include among the controls some with these conditions. The controls consisted of one normal African in apparent good health with no detectable abnormality (# 9); two African patients, one normal except for the presence of ascariasis (# 10), the other normal except for ascariasis and ancylostomiasis (# 11); and three whites in normal condition, (# 12, 13 and 14). There was difficulty in finding other African controls without symptoms of disease.

Plasma obtained from oxalated fresh venous blood was deproteinized with trichloracetic (Christensen *et al.*, 1946). The protein-free filtrate was used for all of the chemical determinations. The total N.P.N. was determined by the micro-Kjeldahl method (Wagner, 1940). The total (NH_2)N was determined by the Sahyun method (1939) utilizing a Beckman spectrophotometer for photometric measurements. For determination of the individual amino acids, the protein-free filtrate was neutralized, then dried in vacuo at 45°C. and the residues redissolved in 10% isopropyl alcohol. The identification of the amino acids was

TABLE 1

Clinical information

PATIENT	CONDITION	APPROXIMATE AGE	STAGE & DURATION	SEROLOGY KHAN	COMPLICATION	TREATMENT	SAMPLE	
							Number	Date
1	Severe sabreshin. Severity: mild	32	Late yaws	Regular: 1+. Quantitative: 4 K.U.	Stool: ascariasis. Moderate blood loss (severed artery)	Penicillin 1 cc. daily, June 18–25	1a 1b	6/18/50 8/ 1/50
2	Early bone involvement, skin ulceration. Severity: mild	11	Tertiary yaws	Regular: ±. Quantitative: 240 K.U.	Stool: ancylostomiasis	None	2a	7/15/50
3	Skin lesion. Severity: mild	4	Primary yaws. Two months	Regular: −.	Stool: ascariasis. Splenic enlargement	None	3a	7/15/50
4	Fulminating skin lesions. Severity: great	15	Secondary yaws. Seven months	Regular: 3+. Quantitative: 40 K.U.	Stool: ascariasis	Penicillin 900,000 U., July 10	4a 4b	7/10/50 8/15/50
5	Very active bone involvement. Severity: moderate	28	Late yaws	Regular: 1+. Quantitative: 480 K.U.	Stool: ascariasis	S.T.B.* Aug. 5–9, Sept. 4–8	5a 5b 5c 5d	7/27/50 8/15/50 8/28/50 9/15/50
6	Active bone involvement. Severity: moderate	25	Late yaws	Regular: 2+. Quantitative: 4 K.U.	Stool: ascariasis	S.T.B., Aug. 5–9	6a 6b	7/21/50 8/28/50
7	Bone involvement. Severity: great	24	Late yaws. Three years	Regular: 1+. Quantitative: 400 K.U.	Stool: ascariasis	S.T.B. Sept. 2–7, Sept. 18–23	7a	8/17/50
8	Early bone involvement, skin ulceration. Severity: moderate	25	Late yaws	Regular: 2+. Quantitative: 240 K.U.	Stool: ascariasis. Malaria smear positive	S.T.B., Sept. 7–12	8a	8/15/50

* S.T.B. refers to a daily oral intake of 20 mg. per kg. of body weight of 4-oxy-3-acetoaminophenyl arsenoxide, a derivative of acetarsone (Friedheim, 1949). It was supplied by courtesy of Endo Products Corp.

made on the isopropyl alcohol extract by the partition chromatographic method of Consden, Gordon and Martin (1944). Chromatography was of the descending type. Troughs and cabinet similar to those described by Steward, Stepka and Thompson (1948) were constructed for the purpose. The solvents were phenol-water in the first direction and butanol acetic acid-water in the second direction. The papers were dried by a blast of warm air at 40 to 45°C.

TABLE 2

Nonprotein nitrogen and total free amino acid nitrogen* in blood plasma*

CASE NUMBER	N.P.N.†	$(NH_2)N$†	CASE NUMBER	N.P.N.‡	$(NH_2)N$‡
			Patients		
1a	18.80	3.06	1b	20.08	3.76
2a	19.34	—			
3a	16.56	—			
4a	19.62	2.68	4b	20.64	2.60
5a	20.46	3.15	5b	21.35	3.38
			5c¶	26.24	4.19
			5d¶	27.63	4.28
6a	20.87	3.21	6b	22.14	3.89
7a	18.85	2.75			
8a	25.14	3.30			
			Controls		
9	26.11	3.18			
10	27.23	4.23			
11	28.07	4.14			
12	30.34	5.14			
13	30.17	4.87			
14	29.13	4.46			

* All values are given in mg. per 100 ml. of plasma.
† Before treatment.
‡ After treatment.
¶ Sample 5c was taken 25 days after the first treatment on case #5.
Sample 5d was taken 10 days after the second treatment.

Several chromatograms of each sample were produced. One was used for amino acids identification and the others for quantitative determinations. After identification of the amino acids by their Rf values and spot colors on the first paper, it was used as a guide to cut the other chromatograms in horizontal strips. The strips were extracted separately with hot water. The extracts were concentrated, taken up in isopropyl alcohol as before and rechromatographed. By this technique it was possible to obtain a clear cut separation of all the amino acids in distinct spots without overlapping. The isatin method of Acher, Fromageot and Justisz (1950) was used for the measurement of proline. For the sulfur-containing amino acids, the platinic iodide method of Winegard and Toennies (1948) was used. The concentrations were calculated according to the method

described by Fisher, Parsons and Holmes (1949). The other amino acids were determined by the Moore and Stein procedure (1948) using ninhydrin and stannous chloride for color development. The concentrations were measured with a Beckman spectrophotometer. The figures reported represent the average of two determinations.

TABLE 3

*Concentrations of individual amino acids in blood plasma**

AMINO ACID	CASE NUMBER†										CONTROLS				
	1b	4b	5a	5b	5c	5d	6a	6b	7a	8a	9	10	12	13	14
Alanine	2.4	1.7	1.9	2.0	3.1	3.3	2.1	2.8	1.5	2.9	1.8	2.0	3.9	3.3	3.1
Arginine	1.2	1.2	1.5	1.6	1.9	1.7	1.3	1.6	1.1	1.6	1.2	1.3	2.7	2.1	2.0
Asparagine	0.4	0.3	0.4	0.4	0.5	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4
Aspartic Acid	0.4	0.5	0.3	0.6	0.7	0.6	0.5	0.7	0.6	0.5	0.5	0.6	0.6	0.8	0.6
α-amino-n. butyric	—	—	0.3	0.5	—	—	0.5	0.4	—	—	—	0.4	0.6	0.7	0.5
γ-amino-n. butyric	—	—	0.3	0.4	0.3	0.5	0.5	0.5	—	0.4	0.5	—	0.9	0.5	0.5
Citrulline	—	—	0.4	0.4	0.5	0.4	0.3	0.4	—	0.4	0.4	0.5	0.5	0.4	0.4
Cystine	0.9	1.6	1.4	0.7	0.5	0.5	1.9	0.6	0.9	1.6	trace	0.5	0.4	0.5	0.4
Glutathione	0.7	0.8	1.0	1.1	0.5	0.4	0.8	0.7	0.7	1.1	trace	0.4	0.3	0.4	0.3
Glutamic Acid	1.8	1.1	1.4	1.5	1.7	2.4	1.0	1.6	1.1	1.8	1.4	2.1	3.0	2.9	1.9
Glutamine	2.0	0.7	1.9	2.2	2.3	2.8	1.7	2.0	0.9	1.9	1.8	1.5	2.3	2.7	2.4
Glycine	0.9	0.9	1.1	1.2	1.5	1.7	0.9	1.4	1.1	1.2	1.2	1.4	1.9	2.1	1.7
Histidine	0.7	0.8	0.8	0.8	1.3	1.3	0.7	0.8	0.9	1.3	1.4	1.3	1.4	1.5	1.3
Leucines	1.4	1.2	1.2	1.6	1.8	2.0	1.5	1.6	1.4	1.9	1.6	1.6	2.5	2.1	2.3
Lysine	1.9	1.4	0.9	1.4	1.7	2.1	1.8	1.9	1.5	1.1	1.7	1.3	2.8	2.6	2.5
Methionine	1.0	0.7	0.7	0.7	0.7	0.7	0.9	0.5	0.7	0.7	0.6	0.6	0.5	0.4	0.5
Ornithine	0.4	0.3	0.7	0.6	0.7	0.6	0.4	0.5	0.5	0.6	0.7	0.6	0.8	1.0	0.4
Phenylalanine	1.2	0.7	0.8	0.9	1.2	1.4	0.8	1.1	0.8	0.9	1.0	1.2	1.7	1.5	1.3
Proline	—	0.5	0.8	0.9	1.0	1.1	0.9	1.2	0.7	0.3	0.7	0.8	0.9	0.4	1.0
Serine	—	—	0.4	0.6	0.6	0.5	0.4	0.5	0.5	0.2	0.6	0.4	0.8	0.6	0.4
Taurine	0.6	0.7	0.7	0.7	1.1	1.1	0.9	0.7	0.7	0.6	0.9	1.3	1.8	1.4	1.0
Threonine	1.3	0.9	1.1	1.2	1.6	1.7	0.9	1.4	0.7	1.3	1.2	0.8	2.0	1.9	1.7
Tryptophane	0.7	0.4	0.6	0.6	0.9	1.0	0.7	0.8	0.4	0.7	0.7	0.9	1.1	1.1	0.8
Tyrosine	1.4	0.7	0.9	1.2	0.8	1.2	1.0	0.8	0.7	1.2	1.2	1.3	2.7	1.9	1.4
Valine	1.5	1.6	1.4	1.7	1.9	2.1	1.8	1.5	1.2	1.8	1.4	1.4	2.4	2.4	2.5

* All values are given in mg. per 100 ml. of plasma.

† The above results were obtained from samples taken at the same time as those listed in Table 1.

EXPERIMENTAL RESULTS

The plasma concentrations of N.P.N. and free $(NH_2)N$ are reported in Table 2. It may be seen that the N.P.N. values for the patients before treatment were all lower than those of the controls with a definite tendency to increase toward normal upon treatment. The N.P.N. of the white controls was higher, ranging from 29.13 to 30.34, while that of the Africans ranged between 26.11 and 28.07.

Since these values fall within the normal level and since two of the controls had ascariasis and one had ancylostomiasis in addition, the results suggest that ascariasis and ancylostomiasis are not accompanied by a low plasma N.P.N.

The free $(NH_2)N$ values show the same trend as the N.P.N. One of the controls, # 9, however, had a concentration of 3.18 which was slightly lower than that of patients # 6 and 8 who had not yet received treatment, and definitely lower than that usually considered normal. Values obtained for some of the patients appear quite low. Number 4 was as low as 2.60 and # 7 was 2.75. The condition of these two patients was particularly severe. Number 7 had received no treatment, but a large dose of penicillin, supposed to be the agent of choice (Dwindelle *et al.*, 1946), had been administered to # 4 twenty days earlier with no apparent effect on the $(NH_2)N$ concentration.

The individual amino acids identified in plasma and their concentrations are given in Table 3. The table shows the values obtained for six of the eight patients and five of the six controls. Material difficulties did not permit analyses of the other cases. Twenty five amino acids were identified. In certain cases a few of these amino acids were not found due probably to the fact that they were present in concentrations too minute to be detected. For patient # 9, cystine and glutathione are shown as traces as they could be detected but no quantitative determination was possible.

It should be pointed out that several amino acids which have not been determined previously in human blood plasma were identified and listed. Among these are aspartic acid, cystine, proline and serine. In the controls, the concentrations of these acids ranged from 0.5 to 0.8 for aspartic, from 0.4 to 0.5 for cystine, from 0.4 to 1.0 for proline and 0.4 to 0.8 for serine (in mg./100 ml. plasma).

The table shows considerable variations in the concentrations of the individual amino acids. The non-sulfur-containing amino acids were lower in the yaws cases than in the normal. As the S.T.B. treatment progressed in cases # 5 and 6, there was an increase in the concentration of these acids. The sulfur-containing amino acids showed the opposite trend. They were greater than normal at first and decreased as the treatment progressed.

DISCUSSION

Woodruff and Man (1945), studying the amino nitrogen concentrations in the plasma of 16 normal females and 21 normal males, report an average of 4.23 mg. per 100 ml. of plasma with 3.37 as the lowest value. In the present investigation, all the yaws cases before treatment had a $(NH_2)N$ below 3.37.

Table 2 shows that the N.P.N. and the total $(NH_2)N$ in the patients were lower than in the controls. The African controls, in turn, had lower N.P.N. and $(NH_2)N$ levels than the white controls. There are, however, other factors related to the variation of the amino nitrogen of plasma. For instance, while increases have been noted in leukemia and yellow atrophy of the liver (Hawk *et al.*, 1947) and nephritis (Papp, 1934), a lowering of concentration has been reported in a few schizophrenic patients (Horwitt, 1944). It is also interesting to

note that while insulin administered to schizophrenics raised their level, it decreased it in diabetic cases (Hawk *et al.*, 1947). A lowering of $(NH_2)N$ was noted during paroxysms but not in the latent stages of induced malarial infection (Howard *et al.*, 1946). It has been reported by Krebs (1950) that considerable variations of food have little effect on the $(NH_2)N$ concentration of human plasma but prolonged starvation may lower it. In general, a decrease of the $(NH_2)N$ level has been observed in few pathological conditions.

In the present investigation, the patients were submitted to a thorough examination and only ascariasis, ancylostomiasis and malaria were found as concomitant diseases. From the experimental results on cases # 10 and 11, it was found that ascariasis and ancylostomiasis are not accompanied by a significant decrease in amino nitrogen. The one case with a positive malaria test, # 8, was in the latent stages and had no abnormal temperature during his hospitalization period. It would then appear that the low $(NH_2)N$ is not due to malaria.

It was practically impossible to obtain from the African patients under examination anything more than vague medical histories. Whether or not any of them had a severe disease in the recent past was not revealed. It is doubtful that the patients' diet previous to their hospitalization was adequate and perhaps even that of the African controls was not as high in nutritional requirements as the diet supplied at the hospital, while it was clearly established that the diet of the white controls was good.

In view of the above, it would appear that yaws is accompanied by a disturbance of the nitrogen metabolism reflected in a lowering of the N.P.N. and the total $(NH_2)N$ levels. However since the possibility of long standing nutritional factors has not been eliminated, a definite conclusion cannot be reached without further study.

It was possible to obtain amino acid data on only two (# 5 and 6) of the four patients who were treated with S.T.B. In these cases its use appears to have resulted in a normal plasma amino acid level. Its clinical effect was favorable, as in all the four patients the rebuilding of bone tissue was particularly striking and they were discharged asymptomatic. It is realized, however, that the amount of evidence presented is insufficient to draw more than suggestions on the value of the drug. A complete report on the use of S.T.B. in the treatment of bone yaws is to be submitted later.

It should be pointed out that for years the relationship of yaws and syphilis has been disputed. A comparative investigation of the two diseases as to abnormal nitrogen metabolism may show variations which would aid in differentiation or may prove a closer relationship of *Treponema pertenue* and *T. pallidum* than is known at the present time.

SUMMARY

The concentrations of nonprotein nitrogen and of free amino acid nitrogen were determined in the blood plasma of several African patients afflicted with yaws. The concentrations were significantly lower than those of the controls.

Chromatographic separation of the plasma amino acids was conducted. Twenty

five of them were identified and their concentrations measured. Among the normal amino acids of human plasma were aspartic acid, cystine, proline and serine.

As compared to the controls, the concentrations of cystine, glutathione and methionine were noticeably high in the cases studied while those of most of the other amino acids were low.

The plasma amino acids of the S.T.B. treated patients were restored to normal concentrations.

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THE ISOLATION OF *ALLESCHERIA BOYDII* SHEAR, AN ETIOLOGIC AGENT OF MYCETOMAS, FROM SOIL

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Although a number of different fungi have been incriminated in the etiology of mycetomas (Madura foot), most have been inadequately described or are of questionable pathogenicity. One of the more clearly defined pathogens is the ascomycete, *Allescheria boydii*, whose imperfect form is *Monosporium apiospermum*. The interrelationship of the two forms remained unsuspected for many years.

In 1921, Boyd and Crutchfield (1) isolated from a human case of mycetoma an ascomycete that was considered by Shear (2) to be a new species of *Allescheria* and was given the name *A. boydii*. Prior to this, Tarozzi in 1909 (3) had described an imperfect fungus from a Sardinian case of mycetoma. This organism was named *M. apiospermum* by Saccardo in 1911 (4, 5).

In 1944, Emmons (6) discovered that a subculture of a strain of *M. apiospermum*, isolated by Shaw and Macgregor in 1935 (7), had begun to produce perithecia similar to those described for *A. boydii*. This established conclusively the identity of the two. Such a relationship had been suspected previously, for Catanei and Goinard (8), in describing an imperfect fungus (undoubtedly *M. apiospermum*), which had been isolated from a mycetoma, had stated: "Par ses caracteres morphologiques le champignon isolé appartient au type *Allescheria Boydii* Shear, 1921, que a été trouvé en Amerique du Nord, dans un cas de mycetome du pied."

A. boydii infections have been reported from all continents of the world except Asia and Australia (Table 1). Like many other mycoses, *Allescheria* infections are not confined exclusively to tropical areas, but are to be considered as Temperate Zone infections as well.

Until recently, all isolates of *A. boydii* (Table 2) had originated from mycetomas or, in two instances, from a case of septicemia (22) and a central nervous system infection (31). As shown in Table 2, the case records of *A. boydii* infections usually reveal a history of trauma, whereby contaminated foreign bodies could have been introduced into the wound. This observation would suggest that *A. boydii* occurs in nature as a saprophyte which may exhibit pathogenicity when introduced into the human host. Weight has been added to this speculation by the isolation of *A. boydii* from four[1] samples of soil collected in Williamson County, Tennessee (Ajello and Zeidberg, 32)[2]. The present communication is a more detailed account of the earlier report.

[1] Three additional strains have been isolated since the one reported previously.

[2] Subsequent to this publication, Dr. C. W. Emmons, in a personal communication, told of having isolated *A. boydii* in 1948 from soil collected in Taneytown, Md.

MATERIALS AND METHODS

The soil samples from which these isolations were made had been collected during a search for *Histoplasma capsulatum* in soil from Williamson County, Tennessee. For this study, all samples were collected from farm premises by scooping soil directly into large glass vials which, after sealing with plastic screw caps and labeling properly, were transported to the laboratory.

Samples were examined for the presence of pathogenic fungi by using the method first developed by Stewart and Meyer (33) and later modified by Emmons (34). After thorough mixing, a heaping teaspoonful of soil was placed in a 25 x 150 mm. test tube containing 30 ml. of physiological saline and was stirred vigorously. After standing for one hour, 5 ml. of the uppermost portion of the

TABLE 1

*Geographic distribution of A. Boydii infections (Total 25)**

CONTINENT	NUMBER OF CASES	CONTINENT	NUMBER OF CASES
North America—U.S.A.		*South America*	
Georgia	1	Argentina	2*
Iowa	1	Brazil	3
Kansas	1	Paraguay	1
Maryland	1	Trinidad	1
Massachusetts	1	Virgin Islands	1
Pennsylvania	1		
Texas	1	Total	8
State Unknown	1		
Total	8		
Europe		*Africa*	
Italy	3	Algeria	3
Sardinia (Italy)	2	Tunisia	1
Total	5	Total	4

* There is some question concerning the identity of the organism isolated by Nino, F. L., *et. al.* (1947) in Argentina (9).

supernatant were pipetted off and one ml. aliquots were injected intraperitoneally into each of 5 mice.[3] At the end of two weeks, the mice were sacrificed and portions of their livers and spleens inoculated into duplicate tubes of a neutral, dextrose-peptone agar.[4] The agar tubes were incubated at 25°C. and examined periodically during a period of six weeks.

On the basis of studies by Strauss and Kligman (35), who demonstrated that mice were rendered more susceptible to fungus infections when the organism was suspended in gastric mucin, it was later decided to mix 5 ml. of the soil

[3] Later reduced to 4.

[4] Dextrose—1.0 gm., adjusted to pH 7.0; Neopeptone—1.0 gm.; Distilled water—100.0 ml.; Agar 2.0 gm.

supernatant with 5 ml. of 5% gastric mucin. One ml. aliquot portions then were injected into mice as described above. It was soon learned that the use of mucin necessitated treating the mice for the first week with daily injections of strepto-

TABLE 2

Isolates of A. Boydii reported in the literature

AUTHOR	GEOGRAPHIC ORIGIN OF INFECTION	PROBABLE PREDISPOSING TRAUMA	PHASE ISOLATED
Tarozzi, G. 1909 (3)	Sardinia, Italy	Knife wound in foot or sprained ankle	Imperfect
Raedaelli, F. 1911 (4)	Italy	Kick by horse	Imperfect
Pepere, A. 1913 (10)	Sardinia, Italy	Not given	Imperfect
De Magalhaes, P. S. 1916 (11)	Brazil, S. A.	Not given	Imperfect
[Linhares, D. 1917 (12)]	Same case as (11)		
Montpellier, J. *et al.* 1921 (13)	Algeria, Africa	Laceration of foot	Imperfect
Boyd, M. F. *et al.* 1921 (1)	Texas, U.S.A.	Thorn in sole of foot	Perfect
Montpellier, J. 1924 (14)	Algeria, Africa	Not given	Imperfect
Anderson, C. 1925 (15)	Tunisia, Africa	Thorn in foot	Imperfect
Da Fonseca O. *et al.* 1927 (16, 16a)	Brazil, S. A.	Not given	Imperfect
Delamare, G. *et al.* 1929 (17)	Paraguay, S. A.	Not given	Imperfect
[Pena, R. 1930 (18)]	Same case as (17)		
Gay, D. M. *et al.* 1930 (19)	Massachusetts, U.S.A.	Foot injury playing football	Imperfect
Jones, J. W. *et al.* 1931 (20)	Georgia, U.S.A.	Splinter in foot	Imperfect
Gellman, M. *et al.* 1933 (21)	Maryland, U.S.A.	Foot crushed by wagon wheel	Imperfect
Catanei, A. *et al.* 1934 (8)	Algeria, Africa	Unknown	Imperfect
Shaw, R. M. *et al.* 1935 (7)	Kansas, U.S.A.	Foot lacerated by wagon wheel	Imperfect
Zaffiro, A. 1938 (22)	Italy	Glass splinter	Imperfect
Giornelli, L. 1939 (23)	Italy	Unknown	Imperfect
Nino, F. L. 1941, (24, 24a)	Argentina, S. A.	Thorn in sole of foot	Imperfect
Carrion, A. L. *et al.* 1944 (25)	Virgin Islands	Unknown	Imperfect
Feinberg, R.* 1944 (26)	Fla., Ga. or N. Y. U.S.A.	Unknown	Imperfect
Twining, H. E. *et al.* 1946 (27)	Pennsylvania, U.S.A.	Heel injury	Imperfect
Meyerding, H. W. *et al.* 1947 (28)	Iowa, U.S.A.	Weight dropped on foot	Imperfect
Nino, F. F. *et al.* 1947, 1949 (9, 29)	Argentina, S. A.	Not given	Perfect
Magalhaes, O. De. *et al.* 1947 (30)	Brazil, S. A.	Not given	Imperfect
Benham, R. W. *et. al.* 1948 (31)	Trinidad, B. W. I.	Post operative infection	Perfect

* Feinberg's patient is now living in Georgia and has been examined recently by the writer. His mycetoma is still active and *A. boydii* was isolated again from his foot.

mycin (1,000 units) and penicillin (12,500 units) to prevent lethal bacterial infections.[5]

[5] In the continuing studies, 1 ml. of a dilution of "Combiotic" Pfizer that gives 2,500 units of streptomycin and 1,000 units of penicillin has been used with satisfactory results with mice weighing 25–30 grams.

Up to the present time, 194 soil samples collected from 41 farm premises have been examined.

RESULTS

Three of five mice inoculated with a soil suspension prepared from sample 8E, without gastric mucin, yielded *A. boydii*.

After two weeks of growth on plates of Sabouraud dextrose agar (Difco), this isolate developed a greyish white floccose colony with a brownish grey pigment on the reverse side of the culture (Fig. 1). The fungus produced numerous unicellular conidia on conidiophores that varied considerably in length and bore

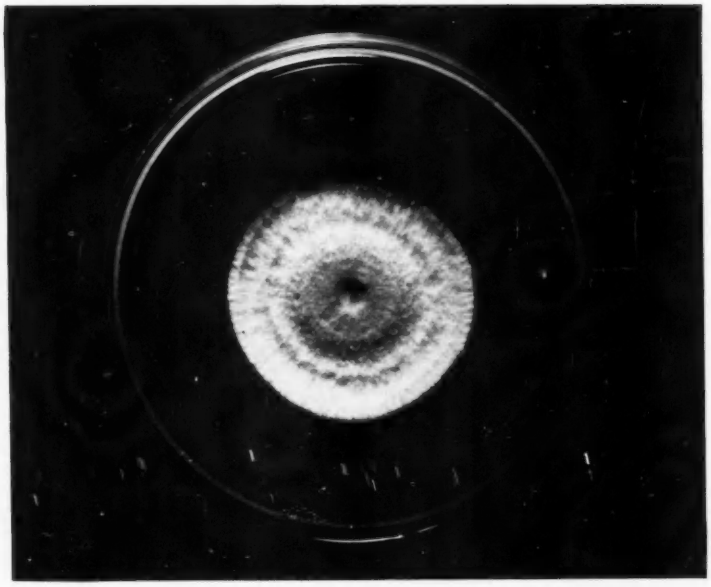

FIG. 1. Appearance of 2 week old colony of *Allescheria boydii* (8E isolate) on Sabouraud dextrose agar.

the acropleurogenous spores singly or in clusters (Fig. 2). The spores averaged 7.3 x 4.4 μ, with extremes of 5.0–11.0 x 3.0–5.0 μ. As noted by Gay and Bigelow (19), and Benham and Georg (31), the type of media in which the fungus was grown greatly influenced conidial size. For example, when grown on tripticase agar at 25°C. the 8E isolate produced spores that averaged 12.2 x 4.7 μ with extremes of 7.5–22.5 x 3.0–5.5 μ. The spores ranged in shape from elliptical to globose to ovate and had a truncate base.

The 8E isolate also produced dark-colored spherical perithecia in abundance (Fig. 3). These cleistogamous reproductive structures ranged from 50 to 90 μ in diameter. When the perithecia were crushed, masses of ascospores emerged

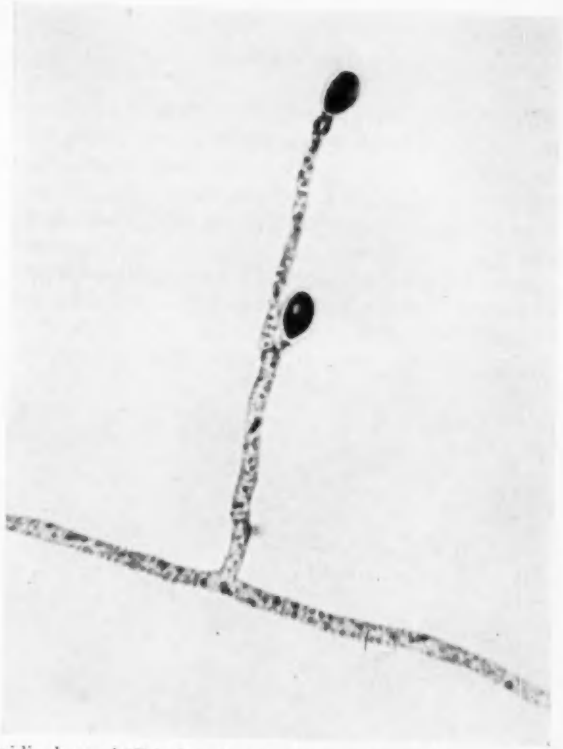

FIG. 2. Conidiophore of 8E isolate bearing a terminal and lateral conidium. × 1, 170

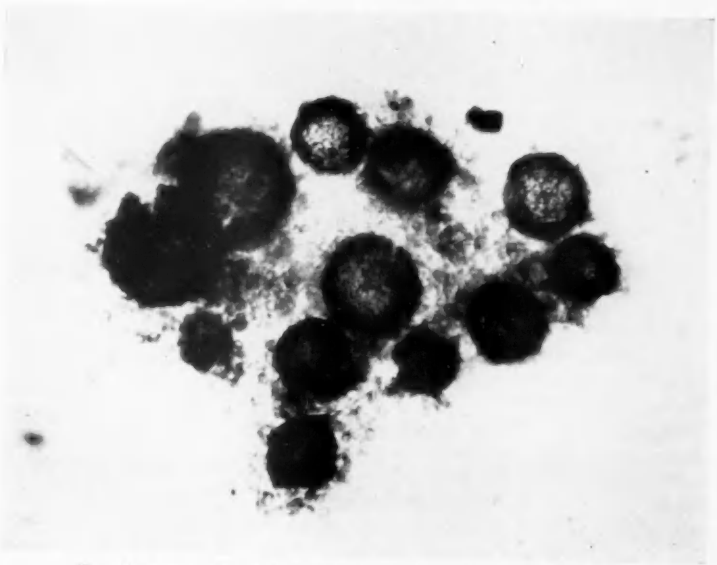

FIG. 3. Group of perithecia produced by soil isolate 8E. × 260

through the ruptured walls (Fig. 4). Due to their delicate nature, very few intact asci were observed. The elliptical ascospores measured 5.0–6.5 x 3.4 μ.

Following the original isolation of *A. boydii* from the 8E soil sample, new saline suspensions were made from this sample and one ml. quantities were injected intraperitoneally into each of 10 white mice. The fungus was isolated successfully from 9 of the 10 mice.

Attempts were made to isolate *A. boydii* from the 8E soil sample by directly inoculating Sabouraud dextrose agar plates with serial dilutions of the soil suspension. All such tests ended in failure.

FIG. 4. Ascospores escaping from a ruptured perithecium. × 618

Using the gastric mucin technique, soil samples 23E, 30E and 31D yielded fungi that were essentially similar to the 8E isolate. However, perithecia were not formed by the 23E isolate.

PATHOGENICITY TESTS

The intraperitoneal injection of mice with spore suspensions from soil isolate 8E and a strain from a human infection failed to produce any gross pathologic changes. There were no deaths. Nevertheless, these fungi were recovered from cultures of the livers and spleens made two weeks after injection.

On the other hand, when the 8E spore suspension was mixed with an equal volume of 5% gastric mucin and injected intraperitoneally into mice, death ensued within one week. No macroscopic pathologic changes were noted in the mice, but histologic examination of the livers and spleens revealed the presence of proliferating mycelial filaments and germinating spores (Fig. 5). The fungus was readily isolated from the internal organs of these mice. A more detailed account of the histopathology of experimental *A. boydii* mouse infections will be reported elsewhere.

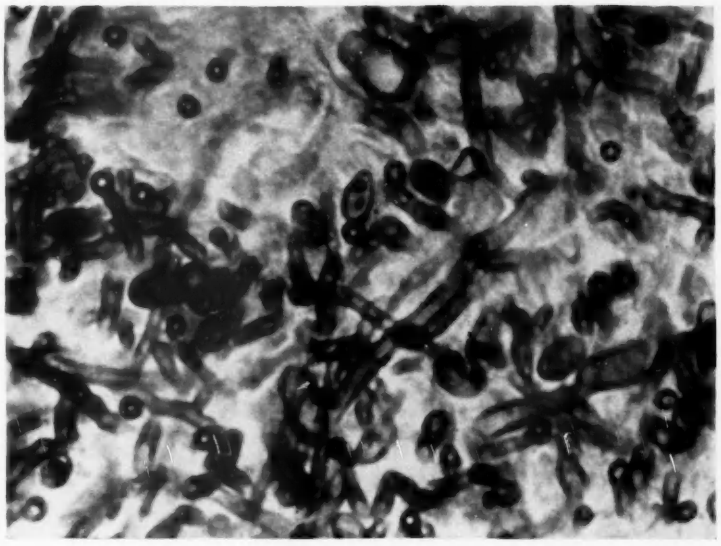

FIG. 5. Section of spleen from a mouse injected with a mixture of gastric mucin and spores of *A. boydii* (8E) showing germinating spores and proliferating mycelium. (Hotchkiss-McManus stain) × 926.

COMMENT

With the passage of time, a growing amount of knowledge has been accumulating regarding the distribution of human pathogenic fungi in nature. Some fungi are components of the normal body flora of many healthy individuals. These may be regarded as endogenous and include such organisms as *Actinomyces bovis*, *Candida albicans*, and *Geotrichum candidum*.

Several species of human pathogenic fungi have been cultured from the environment. The extent of the list of these exogenous organisms is surprising. The 11 different species which have been isolated to date from saprophytic habitats are listed in Table 3.

Others, such as *Blastomyces brasiliensis*, *Hormodendrum compactum*, and most of the dermatophytes (*Microsporum audouini*, *Trichophyton faviforme*, *T. schoenleini*, etc.) have never been isolated from non-living sources.

TABLE 3

Recorded isolates of human pathogenic fungi from non-living sources

ORGANISM	SOURCE	REFERENCE
Allescheria boydii	soil—Maryland	Emmons, C. W. 1948 Personal communication
Allescheria boydii	soil—Tennessee	Ajello, L. *et al.* 1951 (32)
Aspergillus fumigatus	cereal grains, etc.—France and other countries	Renon, L. 1897 (36) and many others
Candida albicans (Aldoi)	decaying vegetables and fruits—Argentina	Negroni, P. *et al.* 1941 (37)
Cryptococcus neoformans	peach—Italy	Sanfelice, F. 1895 (38)
Cryptococcus neoformans	soil—U.S.A.	Emmons, C. W. 1951 (58)
Coccidioides immitis	soil—California	Stewart, R. A. *et al.* 1932 (33)
Coccidioides immitis	soil—California	Davis, B. L. *et al.* 1942 (39)
Coccidioides immitis	soil—Arizona	Emmons, C. W. 1942 (40)
Histoplasma capsulatum	soil—Virginia	Emmons, C. W. 1949–50 (34, 41)
Histoplasma capsulatum	soil—Tennessee	Ajello, L. *et al.* 1951 (32)
Microsporum gypseum	soil—?	Mandels, G. R. *et al.* 1948 (42)
Nocardia asteroides	potato scab—New York	Gordon, R. E. 1946 (43)
Nocardia asteroides	soil—?	Emmons, C. W. 1949 (34)
Phialophora verrucosa	wood pulp—?	Melin, E. *et al.* 1934 (44) (Conant, N. F. 1937) (45)
Sporotrichum schenckii	tree bark, equisetum and oat grains—France	De Beurmann, L. *et al.* 1908 (46)
Sporotrichum schenckii	wheat husks—France	Sartory, A. 1915 (47)
Sporotrichum schenckii	sphagnum moss—?	Emmons, C. W. cited by Gastineau, F. M. *et al.* 1941 (48)
Sporotrichum schenckii	soil—?	Emmons, C. W. 1949 (34)
Sporotrichum schenckii	mine timbers—S. Africa	Brown, R. *et al.* 1947 (49)
Trichophyton mentagrophytes	dung—England	Muende, I. 1937 (50)
	Questionable Isolates	
Blastomyces dermatitidis	wood—Illinois	Stober, A. M. 1914 (51)
Candida albicans	wood pulp—Japan	Taniguchi, Y. 1927 (52)
Candida albicans	carrot—Italy	Verona, O. *et al.* 1938 (53)
Epidermophyton sp.	shoes—Michigan	Jamieson, R. C. *et al.* 1937, 1941 (54, 55)
Epidermophyton sp.	shoes—Maryland	Reed, E. L. 1943 (56)
Microsporum sp.	shoes—Michigan	Jamieson, R. C. *et al.* 1937, 1941 (54, 55)
Microsporum sp.	shoes—Maryland	Reed, E. L. 1943 (56)
Trichophyton mentagrophytes	shoes, socks—Michigan	Jamieson, R. C. *et al* 1937, 1941 (54, 55)
Trichophyton mentagrophytes	socks—Syria	Berberian, D. A. 1938 (57)
Trichophyton mentagrophytes	shoes—Maryland	Reed, E. L. 1943 (56)
T. (rosaceum) megnini	socks—Maryland	Reed, E. L. 1943 (56)
T. violaceum	shoes—?	Jamieson, R. C. *et al.* 1937, 1941 (54, 55)

Other publications deal with the isolation of pathogenic fungi from the environment, but the evidence presented is unconvincing. In this category are the reports designated as questionable in Table 3. One such questionable report is that of Stober (51) who isolated a white mould from wood and identified it as *B. dermatitidis*. This report is invalidated by its failure to describe the microscopic features or to demonstrate pathogenicity in animals.

The several records of the isolation of dermatophytes from shoes and stockings are difficult to evaluate (54, 55, 56, 57). Inadequate descriptions, casual reporting of such exotic species as *T. violaceum* and *T. megnini*[6] (*rosaceum*) (which rarely, if ever, cause tinea pedis), coupled with the investigators' inability to identify suspected isolates of the genus *Microsporum* or the monotypic genus *Epidermophyton*, leave the validity of these findings open to question.

Reports of the isolation of *C. albicans* from wood pulp by Taniguchi (52) and from decayed carrots by Verona and Ciferri (53) cannot be accepted, since the authors' morphologic and physiologic descriptions of the isolates are not typical of *C. albicans*.

SUMMARY

Four strains of *Allescheria boydii* have been isolated from soil collected in Williamson County, Tennessee. A spore suspension of one of these strains when mixed with gastric mucin was found to be pathogenic for mice. A tabulation of all recorded isolations of *A. boydii* has been presented and the habitats of other human pathogenic fungi have been discussed.

ACKNOWLEDGMENT

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UNUSUAL FINDINGS OF FILARIAL INFECTIONS IN MAN

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This is a report of four unusual findings of filarial worms in man. The first case is apparently unique, since it provides information on stages of a filaria that develop typically only in the mosquito intermediate host but are herein reported from man. The second refers to the presence of a filaria in an unusual visceral focus. The third and fourth cases each describe a filaria recovered from a superficial nodular growth.

POST-MICROFILARIAL STAGES IN HUMAN BLOOD FILMS

The patient, a 60-year-old white female, native of Palestine, with a history of 9 years residence in Puerto Príncipe, Haiti and subsequently 11 years in Cartagena, Colombia, was seen by Dr. Moisés Agosín in mid-August, 1950, when the patient was visiting relatives in Santiago, Chile. For approximately 20 years, beginning with her residence in Haiti, she had been afflicted with recurrent edematous erysipelas of both legs and during the last 15 years also of the hands and forearms. Physical examination indicated that she was a well-nourished individual, fairly obese, weighing 90 kilos, with regular pulse of 90 beats per minute, blood pressure of 145/80 mm. Hg., and was afebrile. Both forearms and hands showed symmetrical erythema, desquamation and non-pitting edema of moderate degree. A similar elephantoid type of edema was demonstrated for both legs and feet. There was no evidence of regional lymphangitis or lymphadenitis. Differential white blood count August 18 and 19, 1950 revealed respectively 11 and 8% eosinophilia. (The patient had been examined previously in Haiti, Colombia and New York City without a positive etiologic diagnosis.)

On clinical suspicion that latent filariasis might possibly be demonstrated, thick-blood films were obtained under aseptic conditions. Fifteen films made at 10 a.m. (August 19) were negative for parasites, but preparations made at 2 a.m. the following morning revealed several objects which were at first diagnosed as microfilariae. Careful examination by the senior collaborator, who was in Santiago at the time and saw the patient, provided evidence that the objects

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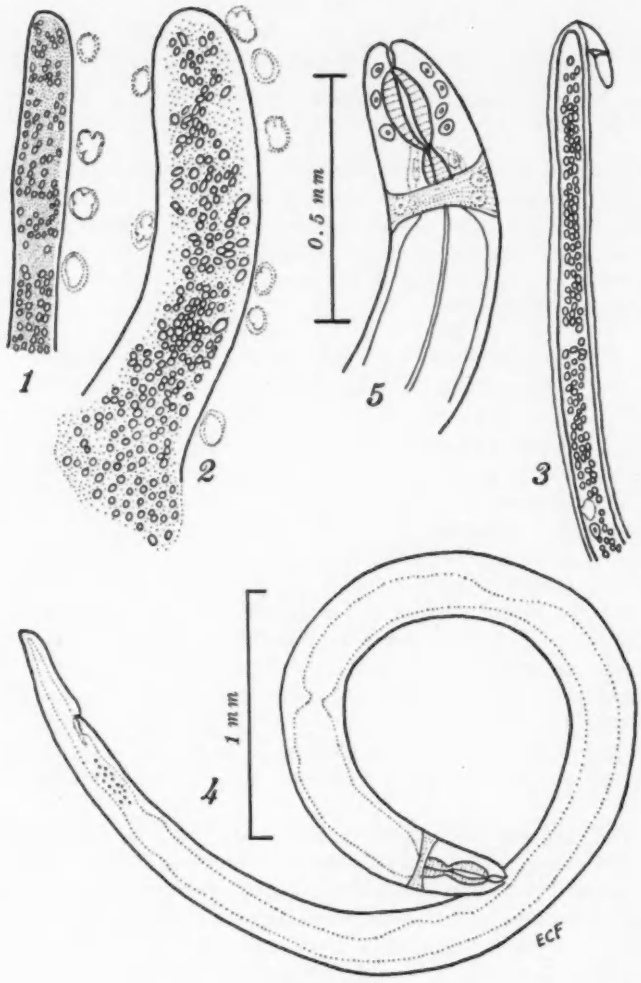
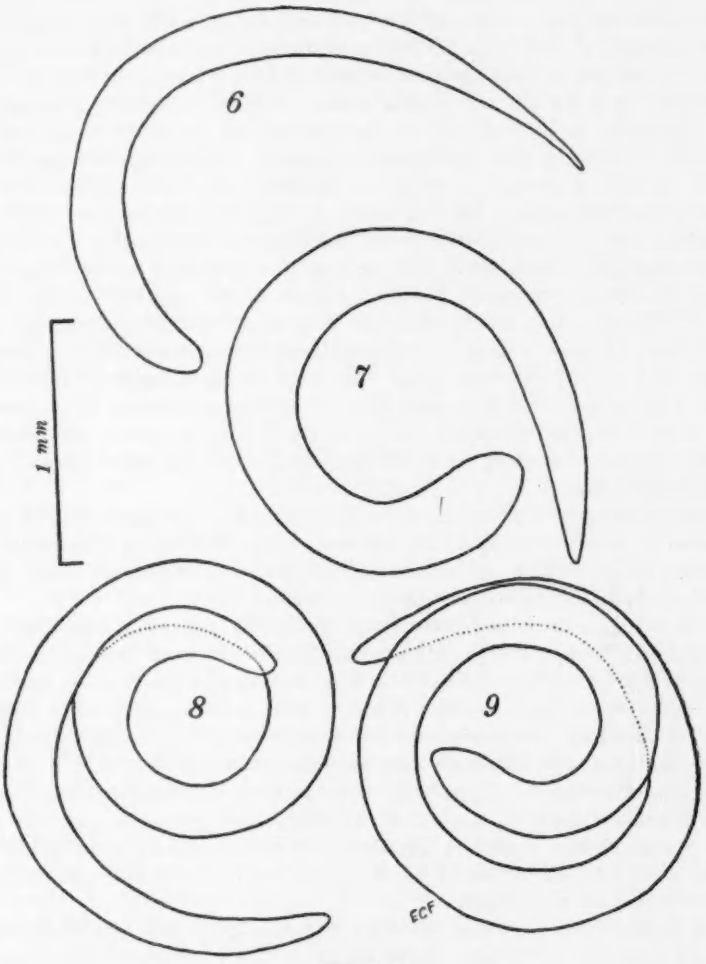


FIGS. 1-5

FIGS. *1–9*. Post-microfilarial stages of *Wuchereria bancrofti* in patient's peripheral blood (Giemsa-strained thick films). *1*, *2*, pre-larval stages; *3*, "sheathed" microfilaria of *W. bancrofti* from Puerto Rican patient, for comparison; *4–9*, developing and mature rhabditoid larvae, *4*, showing digestive tract and nerve ring, *5*, anterior end of *4*, enlarged to show mouth cavity, muscular anterior portion of esophagus, proximal segment of non-muscular portion of esophagus, and nerve ring separating these two parts of the esophagus. Figs. *1*, *2*, *3* and *5* are drawn twice as large as *4*, *6–9*. For comparison, several polymorphonuclear leukocytes and monocytes are outlined adjacent to figures *1* and *2*.

were not typical microfilariae but were apparently more advanced stages of a filaria.

The patient was unwilling to provide additional blood samples and insisted on immediate chemotherapy. Hetrazan was prescribed, 50 mgm. three times daily for 7 days. Post-treatment studies were not possible since the patient returned shortly to her home in Colombia.



FIGS. 6–9

Description of the worms found in thick-blood films (Figs. 1, 2, 4–9).—In wet-drop preparations the night's blood contained nematodes of microscopic size which were sluggishly motile. Slight pressure of the coverglass proved them to be extremely friable. Unusual fragility was also evidenced in the Giemsa-stained

preparations, in which none of the worms most nearly like microfilariae (Figs. 1, 2, 3) were unruptured. Morphologically they resembled microfilariae in most respects but were considerably larger. More mature nematodes in the films proved to be rhabditoid-type larvae (Figs. 4–9).

The microfilaria-like stages in the stained thick films were invariably ruptured in a transverse plane; only anterior and median segments were recognizable. The youngest of these (Fig. 1) had a diameter of approximately 11 microns, contained a number of cells in a densely stained columnar matrix, and was enveloped by a distinct cuticle which had a finger-like contour at its anterior tip. The next size observed was about 16 microns in cross section and had essentially the same internal structure. A somewhat more advanced stage (Fig. 2) had a diameter of about 21 microns and revealed transverse cuticular striations in an equatorial fragment. None of these objects possessed a "sheath." (Compare with Fig. 3, microfilaria of *Wuchereria bancrofti*.) All of the other worms found in the blood films were developing and mature rhabditoid larvae (Figs. 4–9), several of which were intact. The least mature larval stage found (Fig. 6) was about 0.32 mm. long and 22 microns in greatest diameter; a slightly more mature larva (Fig. 7) was about 0.40 mm. long, and a somewhat larger specimen (Figs. 8, 9), about 0.60 mm. long. The most advanced stage present in the films (Fig. 4) had a length of 0.66 mm., a maximum diameter of 26 microns, and a complete digestive tract. Its anterior end (Fig. 5) had a well-developed anterior portion of the esophagus joining a non-muscular posterior portion, and a distinct nerve ring.

The question arises, were these nematodes actually in the patient's peripheral blood or were they contaminants (Manson-Bahr, 1951)? Since the blood was obtained under strictly aseptic precautions, the only conclusion which seems tenable is that they were genuine hemo-parasites.

Similar stages of roundworms have apparently not been described from mammalian blood, but they closely resemble the stages of *Wuchereria* in the mosquito host (cf. Feng, 1936, Pl. I, Figs. 5–8, development of *W. malayi* in *Anopheles hyrcanus* var. *sinensis*). It is concluded that the nematodes described from the blood of this patient are the immediate post-microfilarial and early rhabditoid stages of a filaria that have developed precociously in the definitive host. This development conceivably occurred when microfilariae from visceral blood became trapped in the skin of the patient and proceeded part way with their larval growth. Moreover, Professor Henri Galliard, on learning verbally from the senior collaborator of our finding, observed that these stages in the mosquito host are very fragile.

The patient must have had abundant opportunity in Haiti and Colombia to contract Bancroft's filariasis, hence the most likely etiologic agent was the species *bancrofti*.

FILARIA IN PULMONARY BLOOD VESSEL

A 34-year-old male Colombian Negro entered Hospital San Juan de Dias, Bogotá, on March 31, 1948, with a history of pain in the epigastrium and ten

days later, at the time of his hospital admission, with a painful enlargement in the right cervical region. On examination he was found to be asthenic, febrile, had exquisite tenderness in the region of the epigastrium and a very painful, enlarged, hard, nonmoveable, right cervical lymph node. Both liver and spleen were moderately palpable, and there were signs of pulmonary congestion, with persistent cough and purulent sputum. The blood picture indicated a moderate degree of anemia; the total white count was 47,000, with relative neutropenia and marked lymphocytosis. The patient died the following morning.

At autopsy, performed by Dr. Alberto Garcia-Laverde, there was evidence confirming the tentative clinical diagnosis of acute lymphoid leukemia. Additionally, in the upper lobe of the right lung there was an area of consolidation, in the midst of which there protruded a thread-like nematode, which on section was found to reside in a dilated small artery (Fig. 10). The diameter of the worm ranged between 0.4 and 0.5 mm. The cephalic and caudal extremities were not demonstrated. Histologic preparations (Fig. 11) indicated that a single worm was coiled back and forth in the blood vessel. Two of the cross sections through the uterine coils of the worm contained embryonating eggs.

Although criteria for specific diagnosis are lacking, there is evidence that the worm is a mature female filaria, not improbably *Wuchereria bancrofti*, which is endemic in the area of Buena Ventura, of which the patient was a native.

FILARIAS FROM SUPERFICIAL NODULES

The first of the two filarias reported under this heading was recovered from a cyst removed by Dr. W. Y. Sayad on January 11, 1951 from the left upper eyelid of a 19-year-old white girl, resident of West Palm Beach, Florida. Dr. V. M. Johnson, Pathologist at the Good Samaritan Hospital of this city, reported that the specimen was an ovoidal encapsulated mass, attached to a small elliptical portion of skin. The nodule, which measured 11 by 8 by 7 mm., was reddish gray, and firm but rubbery. Within the nodule there was a thread-like structure, grossly resembling a hair-worm, about 50 mm. in length and coiled irregularly on itself. Histologic section of the nodule indicated that in its center there was chronic inflammatory reaction of a granulomatous type, with a central cleft, in which the foreign thread-like object resided. The lesion was diagnosed "granulomatous pseudocystic reaction, secondary to invasion of a parasitic worm."

A single, delicate nematode-like object and a portion of the nodule were submitted to the senior collaborator for study. The worm proved to be the anterior end of an immature female filaria (Figs. 12, 13), with structural characters at the anterior extremity which place it in the genus *Dirofilaria* (see Chitwood and Chitwood, 1938). The maximum diameter of the specimen was 0.33 mm. and the diameter at the level of the vulvar opening (Fig. 12) 0.16 mm. In view of its morphologic characters and its location in the host's tissues, this filaria is regarded as an example of *Dirofilaria conjunctivae* (Addario, 1885) Desportes, 1939–1940.

The second filaria from a superficial nodule was submitted to the senior

collaborator on August 2, 1951 by Dr. Nelson A. Murray, Consulting Pathologist, Jacksonville, Florida. It was extracted from a small lump on the arm of

Fig. 10

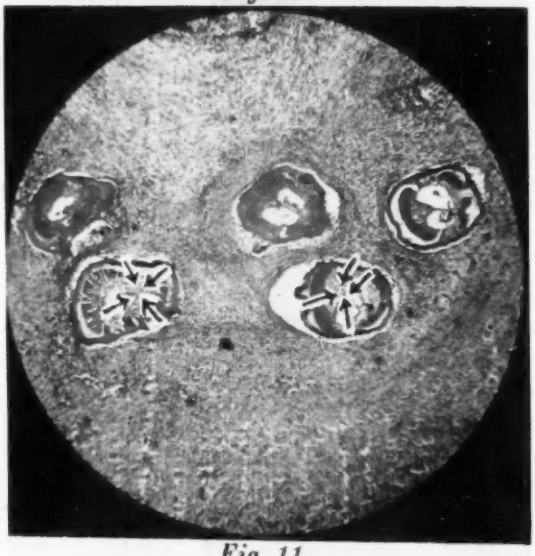
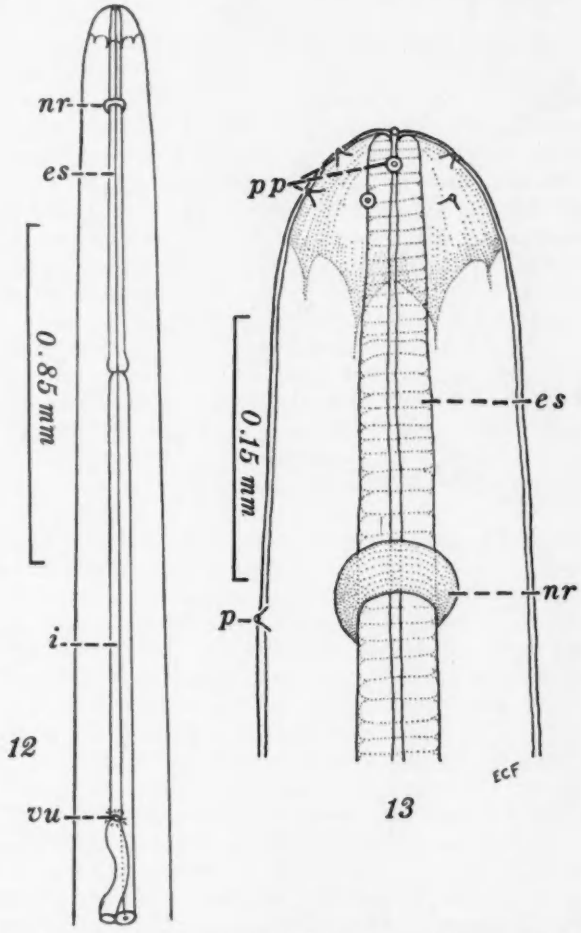


Fig. 11

FIGS. *10*, *11*. Mature female filaria (*Wuchereria bancrofti*?) from pulmonary arteriole. *10*, photograph of cut section in area of pulmonary consolidation, showing loops of worm protruding from the dilated vessel, × 2; *11*, histologic preparation of the lesion with views of the filaria at five different levels, × 40. Arrows in figure *11* point to cross sections of uterus containing eggs.

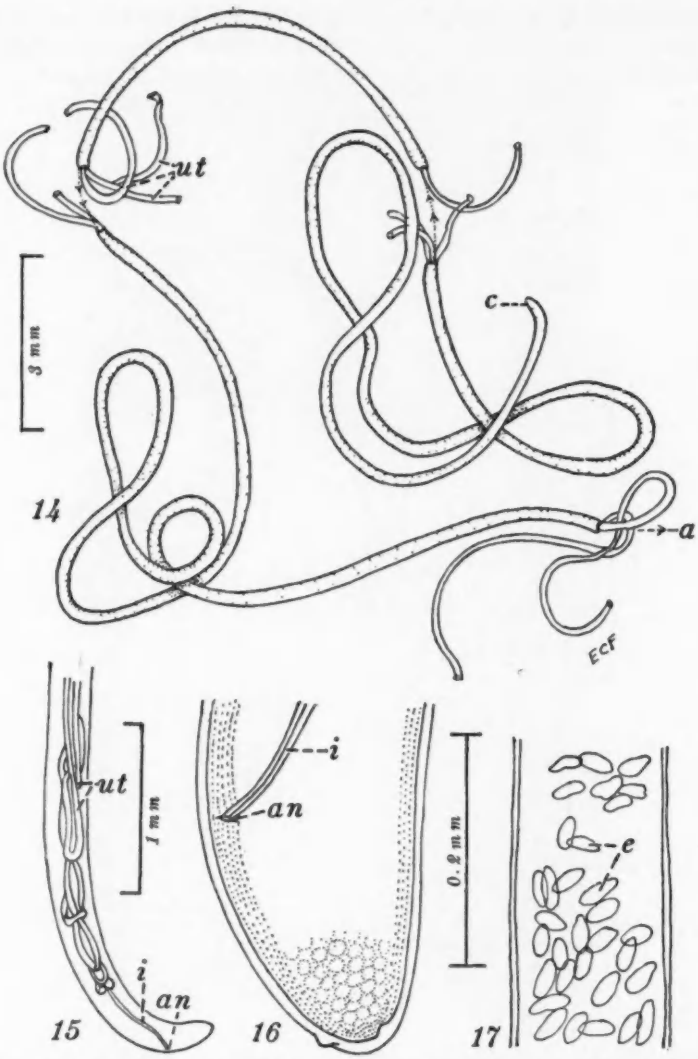
a 35-year-old white woman, living in Jacksonville, following cutaneous incision over the site of the lesion, and was actively motile at the time of removal. The

specimen submitted for diagnosis consisted of three separate adjacent segments of a female filaria, from each of which there protruded eviscerated portions of uterus, with broken ends (Fig. 14). One segment was the caudal most portion



FIGS. *12*, *13*. Anterior end of immature female *Dirofilaria conjunctivae* (Addario, 1885) Desportes, 1939–1940, obtained from nodule on eye-lid of Florida patient. *es*, esophagus; *i*, intestine; *nr*, nerve ring; *p*, cervical papilla; *pp*, peri-oral papillae; *vu*, opening of vulva.

(*c*), while the other two constituted the remainder of the worm except for a portion extending from just behind the vulvar opening to the anterior end (*a*). The total length of the three portions was 69 mm. and the maximum diameter, 0.26 mm. The posterior end in lateral view is enlarged in figures 15 and 16 and a



FIGS. *14–17*. Posterior and middle portions of mature, infertile female *Dirofilaria conjunctivae*(?), removed from nodule on arm of Florida patient. *14*, three adjacent portions recovered; *15*, posterior end of worm; *16*, enlarged caudal tip; *17*, section of uterus containing unfertilized eggs. *a*, arrow pointing to anterior portion, not recovered; *an*, anus; *c*, caudal tip; *e*, eggs *in utero; i*, distal end of intestine (rectum); *ut*, segments of uterus.

short segment of uterus, partially filled with unfertilized eggs, in figure 17. The worm is demonstrated to be a mature, infertile female filaria.

This filaria, like the one obtained from the eyelid of the other Florida patient, is considered to be a specimen of *Dirofilaria conjunctivae* (Addario, 1885) Desportes, 1939–1940.

DISCUSSION

The most interesting of the four cases which we have reported concerns the patient with chronic elephantoid swelling of the extremities, in whose peripheral blood development of post-microfilarial stages of *Wuchereria* has been demonstrated. To explain this finding it is necessary to assume that a living parturient female (or females) existed at the time the patient was seen in Santiago, since it is inconceivable that the stages recovered from the blood could have remained alive for more than a few weeks. Moreover, it seems probable that these filariae reached peripheral blood as microfilariae, otherwise the more advanced stages would have been too large to pass the pulmonary capillaries. Moreover, if any number of these young worms had become lodged in the lungs, there should have been symptoms of pneumonitis. No such evidence was indicated in the patient's history or by physical examination. It is suggested that the microfilariae reached the skin and became lodged there as a result of its pathologic condition, and that some of those which were trapped proceeded with their development to the stages obtained in the blood films. Had opportunity been given for additional study of the patient, more data might have been obtained. The uniqueness of our finding should interest other workers to search for similar stages in their filaria-infected patients.

The filaria lodged in a small pulmonary artery is in an ectopic location. Had this mature female been larger, it might have been considered to be a *Dirofilaria* which had developed in the right heart and had been carried into a pulmonary artery (cf. de Magalhães, 1887, Faust et al., 1937). However, without recovery of a male and without even an opportunity to examine the anterior extremity of the filaria removed from the lesion, no determinative genus or species diagnosis is possible. On epidemiologic grounds, namely opportunity for exposure to filarial infection, the most plausible conclusion is that the filaria is a female specimen of *Wuchereria bancrofti* that had escaped entrapment in a lymph node, had been carried through lymphatic vessels into the peripheral venous circulation and, *via* the right side of the heart, into a small pulmonary artery, where it became lodged and set up considerable tissue reaction.

The two cases of filariae lodged in peripheral nodules are not unique, but are interesting because of the discovery of this infection for the first time in the United States, within a few months of one another in two localities on the east coast of Florida, and because the second of the two worms is a mature, but infertile female. Although specific diagnosis requires examination of the posterior end of the male filaria, the anterior end of the female worm may provide generic characterization; in the first of these two cases the shape of the cephalic tip and the number and distribution of the anterior papillae indicate that this worm is a *Dirofilaria* (Chitwood and Chitwood, 1938). In the second case the anterior end was not recovered; only the caudal end was available for study. Neverthe-

less, in one or more morphologic features, as well as in the location of these female worms in deep cutaneous nodules of man, they conform to the criteria set up by Desportes (1939–1940) for *Dirofilaria conjunctivae* (Addario, 1885).

The species "*conjunctivae*" does not have exact taxonomic status because male worms have never been recovered. This species concept is based on a number of immature female filariae which have been obtained from superficial nodules of persons in Italy, U.S.S.R., Indo-China, Brazil and Argentina. Desportes (l.c.) regards this aggregation as being very close to *D. repens* Railliet and Henry, 1911, which occurs as an adult in the subcutaneous connective tissue of the dog. He separates *D. conjunctivae* biologically from *D. immitis* because the latter is cardiophilic. However, man is probably not the normal host of *D. conjunctivae* for the following reasons: (1) only females have been recovered, (2) its sporadic occurrence would not be sufficient to sustain its life cycle, and (3) females without males would presumably terminate the cycle. Furthermore, the fact that invariably heretofore the worms which have been studied are immature indicates that they are in a suboptimal host. We agree with Desportes (l.c.), that the female filarias allocated to the species "*conjunctivae*" belong to the genus *Dirofilaria*, but we are by no means certain that they constitute a distinct species. Since *D. repens* is not reported for the United States, it seems improbable that the two specimens from Florida can be referred to this species. May these filariae not be *D. immitis* in an inappropriate host? The infective-stage larvae, like those of *Ancylostoma braziliense* (canine or feline strain in man), may have penetrated down to the stratum germinativum but have been physiologically unadapted to enter cutaneous blood vessels of man, subsequent to which they might have been enabled to proceed with their normal development had they been in their suitable definitive host. In *A. braziliense*, the larvae continue to migrate through the deeper layers of the skin, producing "larva migrans." In the case of these filariae, the larvae at first may have migrated within the skin but then became immured by host-tissue encapsulation. This would explain why Dirofilariae which are normally found as adults in the right heart chambers of the dog and its wild relatives, are from time to time recovered only as immature or unmated females in nodules of the human skin.

SUMMARY

1. Four unusual cases of filarial infection are reported from man.

2. In one case, believed to be unique, peripheral night's blood of a Palestinian patient revealed post-microfilarial embryos and rhabditoid larvae of a filaria considered to be *Wuchereria bancrofti*, stages which are characteristically found only in the body of the mosquito intermediate host. It is suggested that microfilariae which reached the skin from a visceral focus became trapped there and proceeded with their development and metamorphosis.

3. In a second case (Colombia) a single fertile female (*W. bancrofti?*) was recovered at necropsy from a small pulmonary artery.

4. In two cases single filariae were obtained from deep cutaneous nodules, one from the upper eyelid of a patient at West Palm Beach, and one from the

arm of a patient at Jacksonville, Florida. The former worm was an immature female but study of the anterior end indicated that it belongs to the genus *Dirofilaria* and is apparently a specimen of *D. conjunctivae*. The second worm is a mature infertile female, of which only the middle and posterior portions were available for study; it is regarded as probably a specimen of the same species. It is suggested the *D. conjunctivae* may possibly be *D. immitis* in an inappropriate host.

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COMPARATIVE MORPHOLOGY OF THE SKIN-INHABITING MICROFILARIAE OF MAN, CATTLE, AND EQUINES IN GUATEMALA[1]

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INTRODUCTION

In 1948 the author was assigned to the Onchocerciasis Project of the Pan American Sanitary Bureau in Guatemala, to study the detailed development of *Onchocerca volvulus* in blackflies of the genus *Simulium*. The original intention was to infect laboratory-reared flies and sacrifice them at various time intervals after the infective blood meal in order to obtain the successive stages of microfilarial development. This had already been done in the Belgian Congo by Wanson, Henrard, and Peel (1946), using *Simulium damnosum*, but it soon became apparent that the three species of *Simulium* which commonly bite man in Guatemala would not feed in captivity. Several thousand blackflies which were allowed to emerge in the laboratory were given the opportunity to feed on susceptible human volunteers under a great variety of conditions. Only three of these flies took a blood meal.

The refusal of laboratory-reared flies to bite made it necessary to investigate the feasibility of employing wild-caught flies for the infection experiments. Nearly three thousand anthropophilic simuliids, captured in the zone where human onchocerciasis is endemic, were dissected. Depending upon the place of capture, zero to four per cent of these wild flies harbored nematode larvae in various stages of development. It is interesting to note that approximately the same rate of infection was found in an area near the endemic zone but presumably free of the disease. This finding suggested another, non-human source of filarial infection in the latter area, and led us to survey the local animal fauna both within and without the endemic zone. Skin biopsies were taken from 884 local animals, mostly horses and cattle. Of this number, 12 per cent of the cattle and 12 per cent of the horses were found to be infected with skin-inhabiting microfilariae, superficially similar to those of human onchocerciasis.

At this time data became available (Dalmat, 1949) indicating that *Simulium metallicum*, *S. ochraceum*, and *S. callidum*, the three species which most commonly bite man in Guatemala and which have long been regarded as the vectors of American onchocerciasis, are also avid feeders on several wild and domestic

[1] The studies and observations on which this article is based were conducted by the Laboratory of Tropical Diseases of the National Institutes of Health, U. S. Public Health Service, under the sponsorship of the Pan American Sanitary Bureau in cooperation with the Dirección General de Sanidad Pública of the Republic of Guatemala. The investigation was aided by a research grant from the Division of Research Grants of the National Institutes of Health.

The work reported in this paper was submitted to the University of Michigan by the author as a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

animals, particularly horses and cattle. The fact that these three species of *Simulium* feed freely on horses and cattle as well as man, and that all three of these hosts harbor microfilariae in the skin, presented the possibility that the naturally-infected flies of the area could have acquired their infection from any one of the three sources. It therefore was deemed advisable to make a comparative study of the skin-inhabiting microfilariae of man, cattle, and horses in Guatemala, in order to allow proper interpretation of future studies on the development of *Onchocerca volvulus* in wild simuliid flies.

METHODS AND MATERIALS

Eight horses and eight cows previously found infected during the animal survey were selected for intensive study, together with a heavily infected human volunteer. Thick blood films were made from the ear of each host animal, stained with Delafield's hematoxylin or Giemsa, and examined for the presence of microfilariae. Blocks of tissue were taken from the skin of the neck, ear, and shoulders, fixed in Bouin's solution and sectioned. Numerous skin biopsies were taken from the same regions of the body, and placed in saline solution until the microfilariae wriggled free. Some microfilariae were studied alive, either in saline alone, in egg albumin alone, or in albumin to which was added various *intra vitam* stains. Toluidin blue and brom cresyl blue were found most useful in this connection. Other microfilariae were mixed with a drop of microfilaria-free human blood on a cover slip and fixed, while still wet, in hot alcohol. The latter preparations were stained with Delafield's hematoxylin, Heidenhain's iron hematoxylin, or Giemsa stain without allowing them to dry. The microfilariae found in the fresh or stained preparations were drawn with the aid of a camera lucida and the drawings were then measured. Specific identification of the microfilariae found in the skin of the three host species was established by comparing them with microfilariae taken from the uteri of gravid *Onchocerca* females obtained from subcutaneous tumors (man) or from the ligamentum nuchae (cattle and horses).

RESULTS

As previously mentioned, skin biopsies revealed the presence of microfilariae n 12 per cent of the horses and cattle in the area. In addition, 129 (87.2 per cent) of 148 cattle autopsied at the local abbatoirs were infected with adult males and females of *Onchocerca gutturosa* Neumann, 1910. This substantiates Caballero's report (1945) of 100 per cent infection in eleven cattle examined in Guatemala. Numerous worms were collected from the loose connective tissue surrounding the ligamentum nuchae, by dissection or artificial digestion, and microfilariae taken from the uteri of such worms were identical with microfilariae from the skin.

Three horses were autopsied in the course of the investigation, one of which harbored adult males and females of *Onchocerca reticulata* (Diesing, 1841 (= *cervicalis* Railliet and Henry, 1910), imbedded in the ligamentum nuchae. Microfilariae taken from the uteri of these worms were identical with the forms found in the skin of horses.

Numerous subcutaneous cysts from cases of human onchocerciasis were dissected or digested by artificial gastric juice, and many adult males and females of *Onchocerca volvulus* (Leuckart, 1893) were thus recovered. Microfilariae from the uteri of these worms agreed in all details with microfilariae from human skin.

The three species of microfilariae dealt with herein are not found in the circulating blood. At least five thick blood films were made from each host animal studied. All were negative. That the failure to find microfilariae in the blood was not due to a periodicity phenomenon nor to low concentrations of microfiariae is indicated by the fact that small skin biopsies about two millimeters in each dimension, taken at the same time as the negative blood films, sometimes contained as many as fifty living microfilariae. The microfilariae are not evenly distributed in the skin over various regions of the body. Many biopsies were negative, and it frequently was necessary to pool several biopsies from a given animal in order to obtain enough microfilariae to make satisfactory stained preparations. The location of the microfilariae just below the papillary layer of the dermis, as well as the tendency of the larvae to be found irregularly distributed in "nests", is well shown in sections of the skin (Plates I and II).

The behavior of *Onchocerca volvulus* microfilariae from human skin is quite different from that of the microfilariae of *O. gutturosa* and *O. reticulata* from cattle and horses. When placed in saline the larvae of the latter two species have a rather slow, deliberate undulating motion which continues for as long as 24 hours after removal from the host. The larvae emerge quite slowly from the cut skin biopsy, and on many occasions we have failed to find microfilariae in biopsy preparations examined in the field immediately after removal from the host, whereas the same preparations, when examined on arrival at the laboratory a few hours later, showed numerous microfilariae. On the other hand, microfilariae from human skin (*O. volvulus*) have a rapid undulating motion which makes them apparent within a minute or two of the time the biopsies are placed in saline. These larvae seldom survive more than a few hours in saline solution.

Table 1 shows the range of size of the three species of microfilariae dealt with here. Considerable shrinkage occurs when microfilariae are fixed, and absolute measurements are thus of little value unless the condition of the specimens is indicated. For this reason, the data for fixed specimens and for living microfilariae are given separately in Table 1.

Microfilariae of *Onchocerca volvulus* tend to be larger than those of *O. gutturosa* and *O. reticulata*, ranging from 210 μ to 300 μ while alive. Ochoterena (1930) gave the average length of *Onchocerca* microfilariae from human skin as 300 μ, and this figure has been quoted by many later Mexican and Guatemalan authors, but only one larva of that size was seen in the present series.

The microfilariae of *O. gutturosa* in the present series fall into an intermediate size group. Fixed specimens ranged from 189 μ to 251 μ, which is considerably greater than the range given by Steward (1937) for fixed material (150 μ-220 μ).

The range of size in *O. reticulata* is tremendous, from 179 μ to 266 μ. Most of the specimens studied fell below 220 μ, however, and the species can be said, in

general, to be smaller than either *O. volvulus* or *O. gutturosa*. It is obvious from Table 1 that the overlap in the size range of the three species is such that total length cannot be considered a distinguishing character.

Table 1 also gives the widths of the three species. It will be noted that *O. volvulus* is considerably wider than the other two species. This is an important point in the practical differentiation of that species from the others.

None of these microfilariae has a sheath, but the cuticula of the three types is marked with very fine transverse striations. Since the work of Fülleborn in 1913 it has been appreciated that the differentiation of species of microfilariae rests to a certain extent upon the relative location (distance from anterior end) of certain significant structures. These are: the beginning of the nuclear column, the position of the nerve ring, the excretory pore and excretory cell, the so-called "genital cells" of Rodenwaldt, the anal pore, and the end of the nuclear

TABLE 1

Comparison of length and width of skin-inhabiting microfilariae of man, cattle, and equines

	MEASUREMENTS, IN MICRA, OF THE THREE SPECIES*		
	O. volvulus	O. gutturosa	O. reticulata
Length—Microfilariae alive	263.0 ± 26.7 (210–300)	222.6 ± 15.3 (197–240)	222.9 ± 28.2 (179–266)
Length—Microfilariae fixed	244.0 ± 12.8 (217–254)	227.8 ± 16.1 (189–251)	202.9 ± 14.3 (190–227)
Width—Microfilariae alive	6.5 ± 0.4 (5.7–6.7)	4.3 ± 0.2 (4.2–4.5)	4.4 ± 0.2 (4.0–5.1)
Width—Microfilariae fixed	4.2 ± 0.1 (4.0–4.5)	2.7 ± 1.1 (2.7–2.9)	2.8 ± 1.6 (2.5–3.0)

* For each value, the mean and standard deviation are shown, with the range given in parentheses.

column (see Plate III, Fig. 14). Since microfilariae vary considerably in their total length, the distance of these various structures from the head end is expressed in terms of percentage of total length. The so-called "Innenkörper" of Fülleborn ("central viscus" of Manson), which forms such a conspicuous part of some microfilariae, has not been found in our species.

Table 2 shows the comparative locations of the significant structures. It will be noted that the nerve ring, the G_1 cell, the anal pore, and the termination of the nuclear column are so similarly located in the three species that no distinction can be made. The excretory pore in *O. gutturosa* is slightly more posterior than in the other two species, but the difference is too slight to be of much diagnostic value. Microscopic examination of the microfilariae gives the impression that the clear area in front of the nuclear column is longer in *O. volvulus*, and the data bear this out to a certain extent. As in the other cases, however, the ranges

of variation in the three species overlap sufficiently that the character cannot be considered valid in distinguishing the species.

The form, size, and arrangement of the cephalic and caudal nuclei, however, do present characters which, in conjunction with the greater width of *O. volvulus* already mentioned, allow separation of the three species (See Plate III). Neither vital stains nor Giemsa stain gave precise enough results to bring out the details of nuclear structure. Wet fixation followed by iron hematoxylin stain produced the most satisfactory results, although occasionally Delafield's hematoxylin

TABLE 2

Location of significant structures of skin-inhabiting microfilariae of man, cattle, and equines

	MEASUREMENTS OF THE THREE SPECIES, EXPRESSED AS PER CENT OF TOTAL LENGTH*		
	O. volvulus	O. gutturosa	O. reticulata
Beginning of nuclear column	3.3 ± 0.8 (2.4–4.9)	2.1 ± 0.6 (1.3–3.7)	1.9 ± 1.5 (1.2–3.6)
Anterior margin of nerve ring	24.0 ± 1.2 (21.5–26.2)	23.7 ± 1.8 (19.0–27.4)	23.7 ± 1.3 (22.4–27.1)
Posterior margin of nerve ring	25.7 ± 1.2 (23.0–27.9)	25.2 ± 1.9 (20.1–28.8)	25.4 ± 1.4 (23.7–29.1)
Excretory pore	35.3 ± 1.4 (33.7–36.1)	40.6 ± 2.3 (37.4–42.5)	36.3 ± 2.7 (32.7–39.6)
Excretory cell	40.2 ± 2.3 (37.8–42.4)	44.5 ± 2.8 (40.0–46.9)	—
Nucleus of cell G_1	69.1 ± 2.6 (64.0–74.2)	71.1 ± 3.7 (65.2–80.3)	71.0 ± 1.8 (67.2–73.8)
Anal pore	83.5 ± 2.0 (82.0–84.9)	82.8 ± 2.0 (78.2–85.6)	85.0 ± 2.8 (82.9–91.0)
End of nuclear column	95.9 ± 1.2 (94.0–97.3)	96.0 ± 0.8 (94.5–97.7)	96.6 ± 1.0 (94.5–98.1)

* For each value, the mean and standard deviation are shown, with the range given in parentheses.

was satisfactory. All of the figures of Plate III were drawn from microfilariae stained with iron hematoxylin.

In *Onchocerca volvulus* the two most anterior nuclei are somewhat variable, being arranged either tandem or abreast, but the greater width of this species allows it to be easily distinguished from the others (Figs. 1–2). Microfilariae of *O. gutturosa* have two large anterior tandem nuclei, followed by a single large nucleus in the mid-line of the larva (Figs. 3–4). Those of *O. reticulata* usually have a large, solitary nucleus followed by a tandem pair (Figs. 5–6), but oc-

casionally one is found with the arrangement shown in Figure 7. Such larvae cannot be distinguished from *O. gutturosa* except by reference to the nuclei of the tail, which show distinct differences among the three species. In *O. volvulus*, the pattern consists of two medium-sized terminal nuclei, one behind the other, preceded by one or two discrete, larger nuclei in file (Figs. 8–9). Microfilariae of *O. gutturosa* have a long terminal bar (Fig. 10) which, in favorably stained specimens, is seen to consist of four tiny, discrete nuclei (Fig. 11). This is preceded by a group of rather large, quadrangular nuclei which are more or less confluent (Figs. 10–11). The microfilariae of *O. reticulata* also have a terminal bar, but in our material it is always more delicate than that of *O. gutturosa*, and never becomes resolved into discrete nuclei. It is preceded by a chain of small, distinct, triangular nuclei which rarely become confluent (Figs. 12–13). The entire appearance of the caudal nuclei is much more delicate than in *O. gutturosa*, and it usually is easy to distinguish the two species on that basis. In about 10 per cent of the specimens of *O. reticulata*, however, there is a tendency for the small triangular nuclei to appear larger and more or less confluent. It is difficult or impossible to distinguish such specimens from those of *O. gutturosa*. It is not known whether this appearance represents actual variation in the nuclear structure or is merely the result of faulty staining.

SUMMARY

1. Man, cattle, and horses in the onchocerciasis zone of Guatemala are infected with microfilariae which inhabit the dermal layer of the skin and do not circulate in the blood. These microfilariae belong to the species *Onchocerca volvulus* (Leuckart, 1893), *O. gutturosa* Neumann, 1910, and *O. reticulata* Diesing, 1841 (= *O. cervicalis* Railliet and Henry, 1910), respectively.

2. The microfilariae of these three species can be distinguished morphologically on the basis of the form, size and arrangement of the caudal nuclei.

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PLATE I. Section of the skin of a horse, showing several microfilariae of *Onchocerca reticulata* (Diesing) just below the papillary layer of the dermis.

PLATE I

PLATE II. Section of the skin of a cow, showing a microfilaria of *Onchocerca gutturosa* Neumann in the papillary layer of the dermis.

PLATE II

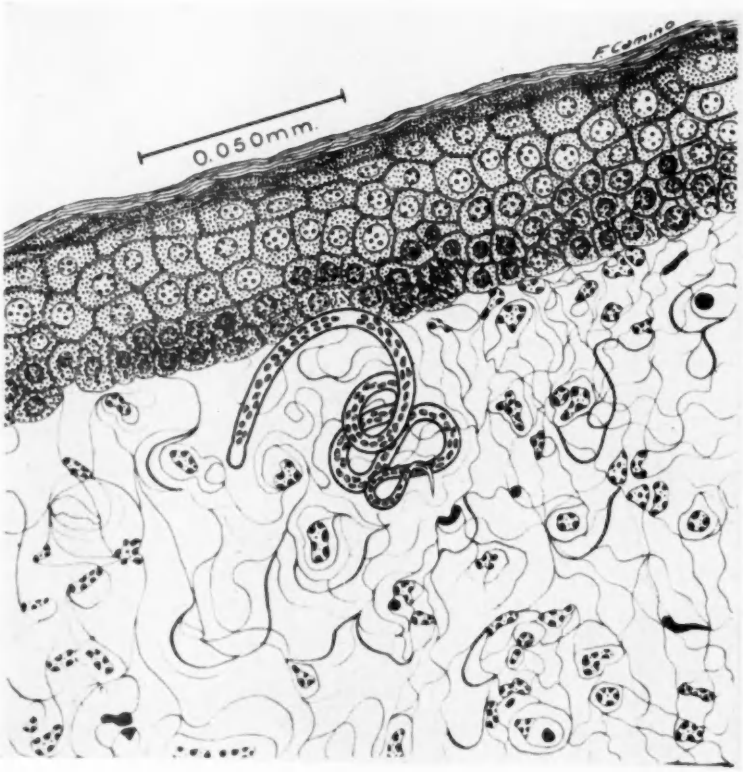
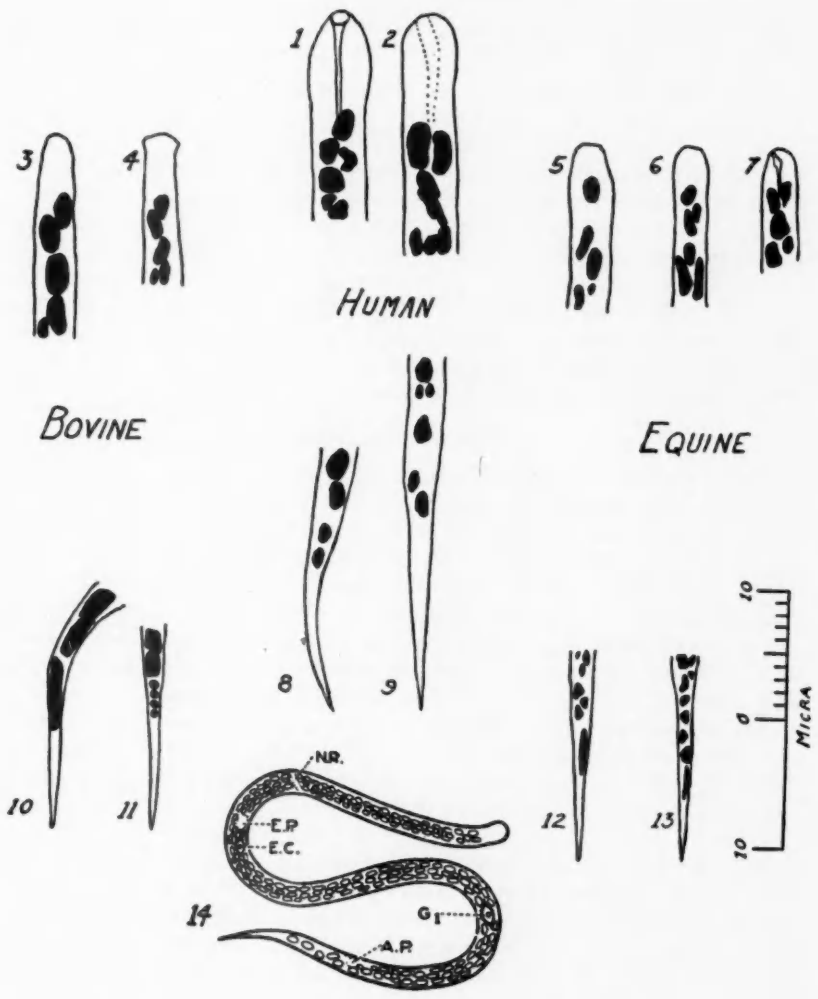


PLATE III. Figs. 1, 2. Cephalic nuclei of *Mf. volvulus*. Figs. 3, 4. Cephalic nuclei of *Mf. gutturosa*. Figs. 5, 6, 7. Cephalic nuclei of *Mf. reticulata*. Figs. 8, 9. Caudal nuclei of *Mf. volvulus*. Figs. 10, 11. Caudal nuclei of *Mf. gutturosa*. Figs. 12, 13. Caudal nuclei of *Mf. reticulata*. Fig. 14. Semidiagrammatic representation of *Mf. volvulus*. (Not drawn to scale.) AP, anal pore; EC, excretory cell; EP, excretory pore; G_1, first "genital" cell; NR, nerve ring.

PLATE III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THE HISTOPATHOLOGY OF EXPERIMENTAL SCHISTOSOMIASIS

I. The Hepatic Lesions in Mice Infected with *S. mansoni*, *S. japonicum* and *S. haematobium*[1]

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INTRODUCTION

As part of a general study of the histopathology of infections with *Schistosoma mansoni*, *S. japonicum* and *S. haematobium* in experimental animals, white mice were infected with cercariae from single snails or several snails in order to produce unisexual or bisexual infections. From some of these mice histological sections have been prepared for the purpose of studying the changes which occur in the liver as the result of the presence of worms in the mesenteric-portal circulation independent of the deposition of eggs. This paper presents our observations on male and female unisexual and early bisexual infections with *S. mansoni* and *S. japonicum*, and male unisexual infections with *S. haematobium*.

REVIEW OF LITERATURE

There is general consensus of opinion that, in addition to the characteristic lesions produced in the liver about eggs of the schistosomes, toxic substances from the maturing worms in the mesenteric-portal veins are the cause of the early clinical manifestations of the infection in man, and some students of the subject believe that similar substances from mature worms contribute to the later development of cirrhosis of the liver and splenomegaly. The fever, giant urticaria and eosinophilia occurring during the third to fifth week after infection and before eggs appear in the excreta apparently represent a systemic reaction to the worms themselves, and coincide with the time when copulation is first taking place between maturing male and female worms. The participation of toxic substances from adult worms in the development of cirrhosis has been emphasized particularly in *S. mansoni* infections because of the paucity of eggs usually found in the liver in extreme cases of so-called "pipe-stem" cirrhosis.

The histopathology of early infections has been studied intensively only in experimental animals. Fairley (1920) infected monkeys with *S. mansoni* and *S. haematobium* and found, in animals dying within 5 weeks of infection, cloudy swelling of the liver parenchyma and displacement of hepatic tissue by focal collections of inflammatory cells. Faust and Meleney (1924) described in a dog, hyperinfected with *S. japonicum* and dying 14 days after infection, extensive "coagulative necrosis" of liver cells about the central veins, sometimes associated

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with hemorrhage, but without cellular infiltration. Koppisch (1937) infected rabbits and white rats with *S. mansoni*. In rabbits he found up to the thirtieth day of infection increasingly dense cellular infiltration about interlobular veins spreading into the adjacent parenchyma, and endophlebitis of interlobular veins, with beginning granulation tissue in a few portal spaces. In rats he found similar cellular infiltrations and in addition small areas of "hyaline necrosis" of the parenchyma. The above infections were all bisexual.

Unisexual infections to study liver pathology in schistosomiasis were first reported by Lee (1932). In 25 Chinese hamsters (*Cricetulus griseus*) infected with cercariae of *S. japonicum* from single snails and sacrificed from 23 days to 2 years and 4 months after infection, he found lymphocytic infiltrations in periportal spaces and occasionally between columns of liver cells in all except one animal, but he discounted this finding because he found similar infiltrations in uninfected hamsters. Even in the late infections, however, no evidence of diffuse or localized fibrosis of the liver was found.

Jaffé, Mayer and Pifano (1945) in Venezuela infected white mice and guinea pigs with *S. mansoni* cercariae from single snails (*Australorbis glabratus*). Among 22 unisexual infections (14 male, 8 female) from one to five months in duration they observed in sections of liver of 3 mice harboring male and 5 harboring female worms foci of necrosis, consisting of "homogenization" of the cytoplasm of parenchymatous cells. The nuclei had sometimes lost their affinity for stains. The foci of necrosis were frequently mid-zonal, often extending to the center of the lobule. In some cases they were isolated, in others confluent. Frequently there was round-cell infiltration at the periphery, in some cases with a little increase of the connective tissue. Similar necrosis had previously been observed in 9 out of 78 animals having bisexual infections. The authors concluded that this necrosis was caused by toxins from the schistosome worms, and that it was an acute manifestation of the process which leads to cirrhosis of the liver in man. They also frequently found "vascular lesions" in the liver in unisexual infections. These consisted of cellular accumulations about portal venules, either partly or entirely surrounding the vessel, in some cases completely occluding the lumen, in others extending outward into the parenchyma. These accumulations were found more often in animals infected for five months than in earlier infections.

Girges (1934) advanced the idea that Egyptian splenomegaly with its attendant cirrhosis of the liver is caused by the toxic action of male worms alone in the branches of the portal vein. His thesis was based upon the finding of large numbers of male worms and few or no female worms in these vessels in human autopsies and in biopsy sections of the liver from clinical cases. His argument is that male miracidia are produced in larger numbers than female miracidia; that the male larval stages in the snail are more resistant to unfavorable conditions than the female; that infection in Egypt occurs primarily in the summer when the canals are almost dry, thus creating unfavorable conditions for the survival of the female larval stages; that the presence of male worms in the human host immunizes him against subsequent infection with female worms;

and that the failure of the male worms to migrate from the liver to the mesenteric vessels in the absence of female worms permits their toxins to act more directly on the liver tissue. Girges also described isolated areas of necrosis in the liver parenchyma surrounded by small mononuclear cells similar to those found in typhoid fever.

Fairley, Mackie and Jasudasan (1930) exposed guinea pigs to large numbers of cercariae of *Schistosoma spindale* from several snails, but recovered only a relatively small number of worms from the portal veins, all being immature males. The absence of female worms was attributed to the unsuitability of the host for their development. In the livers of these guinea pigs endophlebitis and thrombosis of portal venules was found, associated directly with worms in these vessels, often undergoing disintegration. Perivascular infiltration with mononuclear and polymorphonuclear leucocytes was also found, generally associated with periportal fibrosis. Venous congestion, fatty degeneration and "toxic spoiling" of parenchymal cells were also encountered in several animals.

METHODS

For unisexual infections with *S. mansoni*, white mice were exposed to known numbers of cercariae from a single snail (*Australorbis glabratus*) by immersing them individually in glass jars containing water to which the cercariae had been added. In most cases 100 or 200 cercariae were employed but in some cases fewer or more were used. The mice were killed by ether and autopsied at stated intervals from 2 to 70 weeks after infection. Their thoracic and abdominal organs were inspected for macroscopic lesions. Portions of liver and spleen, and in some cases lungs and kidneys, were fixed in Zenker's or Bouin's solution and microscopic sections were stained with hematoxylin and eosin and sometimes with Foot's modification of Masson's trichrome stain for connective tissue. In the later part of the study the remainder of the liver and the mesenteric vessels were perfused by the method of Yolles *et al.* (1947) in order to determine the sex of the worms and the approximate number of worms which had reached the mesenteric-portal circulation. Press preparations of the liver were also examined to eliminate the presence of eggs.

For bisexual infections with *S. mansoni* most of the mice were infected percutaneously, a few intraperitoneally, with known numbers of cercariae from several snails. The mice were autopsied at intervals from one-half to 5 weeks after infection and sections were prepared as above. It was found that at 5 weeks only a few mice with bisexual infections showed evidence of egg deposition in the liver. Only those showing no egg deposition are included in the study.

For unisexual infections with *S. japonicum*, mice were exposed percutaneously to known numbers of cercariae from single snails using 50 to 200 cercariae per mouse, and with *S. haematobium* using 150 to 300 cercariae per mouse. The japonicum mice were sacrificed from 2 to 95 weeks after infection, the haematobium mice from 2 to 65 weeks after infection.

The sex of worms in the early unisexual infections was determined by knowing in advance that the cercariae from the snail employed had previously produced

infections of only male or female worms. If the infections were of long enough duration, the sex was determined by examining the worms recovered from the mice at autopsy.

As a control, tissue sections were prepared from the livers of a number of young and old uninfected mice.

The material studied consists of histological sections of the livers from the following mice:

15 uninfected mice
104 unisexual male infections with *S. mansoni*
71 unisexual female infections with *S. mansoni*
66 bisexual infections with *S. mansoni*
29 unisexual male infections with *S. japonicum*
22 unisexual female infections with *S. japonicum*
4 bisexual infections with *S. japonicum*
17 unisexual male infections with *S. haematobium*

RESULTS

Uninfected mice. In young mature mice the liver may show practically no cellular infiltrations either in the periportal connective tissue or in the parenchyma, but more often it contains small accumulations of mononuclear cells about the portal venules and occasionally larger circumscribed nodular accumulations extending into the liver parenchyma. It may also contain small circumscribed accumulations of mononuclear and polymorphonuclear cells in the parenchyma, apparently not associated with portal venules (Fig. 1). In old mice there is usually more cellular infiltration, and in two of our mice, each about a year old, there was in addition an area in the parenchyma of intense polymorphonucelar infiltration with loss of liver cells and fragmentation of the invading cells (Fig. 2).

Periportal and parenchymal cellular infiltration in S. mansoni *infections.* In unisexual infections significant cellular infiltrations were found in about half of the mice. They increased in incidence and severity during the first 8 to 12 weeks of infection after which they tended to decrease. They varied widely in extent and intensity, and there was no demonstrable correlation with the number of cercariae employed or the number of worms recovered from the mesenteric-portal circulation. There was no significant difference between the lesions found in male and in female infections.

The earliest evidence of excessive periportal cellular infiltration in a unisexual infection was found in a male infection of 2½ weeks duration and in a female infection of 3½ weeks duration. In addition to mononuclear cells there were polymorphonuclears in these accumulations. Although many of the polymorphonuclear leucocytes had cytoplasm which stained red with eosin, the granulation of the cytoplasm was not distinct enough to identify the cells as eosinophiles. There was also an increase in the number and size of parenchymal infiltrations, in which polymorphonucelar cells predominated. By 4 to 4½ weeks a few male and female infections were associated with intense periportal and parenchymal

infiltrations, which encroached upon the parenchymatous tissue producing disintegration of glandular cells (Fig. 3). This type of lesion was found with increased frequency in infections up to 16 to 20 weeks in duration. In older infections the periportal infiltration was usually less intense and tended to become mononuclear in type, and the parenchymal infiltration was usually slight or absent (Figs. 4 and 5). Multinucleated cells with centrally located nuclei were occasionally present in portal spaces having intense cellular infiltration.

The periportal infiltrations sometimes completely surrounded the portal venules, especially in the more intense cases, but often were in the form of nodular accumulations partly surrounding the vessels (Fig. 5). The parenchymal infiltrations were sometimes small and isolated, but in more intense cases were confluent and irregular in shape, and sometimes were continuous with periportal infiltrations.

In mice with bisexual infections up to 5 weeks duration, periportal and parenchymal infiltrations were in general more numerous and more intense

TABLE 1

Periportal cellular infiltration in livers of mice infected with S. mansoni

INTENSITY OF INFILTRATION	TYPE OF INFECTION AND DURATION IN WEEKS							
	Unisexual						Bisexual	
	Number of Mice			Percentages			No. of Mice	Percentage
	2–5 weeks	5½–12 weeks	14–70 weeks	2–5 weeks	5½–12 weeks	14–70 weeks	2–5 weeks	2–5 weeks
None or slight	43	14	13	70.5	26.9	21.0	24	45.3
Moderate	10	13	28	16.4	25.0	45.1	21	39.6
Intense	8	25	21	13.2	48.1	34.0	8	15.1
Total	61	52	62	100.1	100.0	100.1	53	100.0

than in the unisexual infections of similar duration. Significant infiltrations were observed in an infection of 1½ weeks duration, and they increased in frequency and intensity so that at 5 weeks the average severity was comparable to that in unisexual infections up to about 8 weeks in duration (Fig. 6).

The findings with reference to periportal and parenchymal cellular infiltration in *S. mansoni* infections are summarized in Tables 1 and 2. The tables are arranged to show the relation between duration of infection and intensity of infiltration. The unisexual infections are divided arbitrarily into three periods, the first (2–5 weeks) corresponding to the period in bisexual infections before egg deposition begins, the second (5½–12 weeks) during which the most intense infiltrations usually were found, and the third (14–70 weeks) when the periportal infiltrations tended to become smaller and mononuclear in type and the parenchymal lesions tended to disappear. It should be realized that the degrees of intensity of infiltration are subjective impressions without clear-cut demarcation.

In Table 1 it will be seen that, in the unisexual infections of 2–5 weeks dura-

tion, most of the mice showed slight or no periportal infiltration, although a few already showed intense infiltration. In the 5½–12 week period nearly half showed intense infiltration, while in the 14–70 week period the largest percentage showed only a moderate degree of infiltration and some still showed none or a slight amount. The bisexual infections of 2–5 weeks durations showed a considerably lower percentage of insignificant infiltrations and a correspondingly higher percentage of infiltrations of moderate intensity than the unisexual infections of similar duration.

In Table 2 it will be seen that in the unisexual infections the parenchymal cellular infiltrations were, on the average, less intense than the periportal infiltrations, that they tended to increase during the 5½–12 weeks period, and that they were usually absent in the 14–70 weeks period. In the bisexual infections of 2–5 weeks duration the parenchymal infiltrations were, on the average, much more intense than in the unisexual infections of similar duration.

TABLE 2

Parenchymal cellular infiltration in livers of mice infected with S. mansoni

Intensity of infiltration	Type of infection and duration in weeks							
	Unisexual						Bisexual	
	Number of Mice			Percentages			No. of Mice	Percentage
	2–5 weeks	5½–12 weeks	14–70 weeks	2–5 weeks	5½–12 weeks	14–70 weeks	2–5 weeks	2–5 weeks
None or slight	50	25	53	82.0	48.1	85.5	17	32.1
Moderate	5	16	4	8.2	30.8	6.5	17	32.1
Intense	6	11	5	9.8	21.2	8.2	19	35.8
Total	61	52	62	100.0	100.1	100.2	53	100.0

Coagulative necrosis in liver parenchyma in S. mansoni *infections.* In addition to replacement of parenchymatous liver tissue by cellular infiltration, sections of many livers contained characteristic areas of necrosis similar to those described by Jaffé, Mayer and Pifano (1945). These consisted of sharply demarcated areas of parenchymatous cells in which the cytoplasm stained a diffuse bright pink, and the nuclei were shrunken and pyknotic or absent. The areas usually occupied part of a lobule but sometimes extended over several lobules. They often appeared to have no direct relationship to liver lobules, but sometimes were adjacent to portal spaces or central venules. Occasionally these vessels were involved in the necrosis (Fig. 10). The necrotic areas were apparently independent of the areas of cellular infiltration described above. Some were adjacent to portal venules which were obstructed by schistosome worms (Figs. 7 and 8.)

The course of development of these areas of necrosis seemed to be as follows. The original insult to the area seemed to have occurred at one time because of the uniform appearance of the cells and the sharp demarcation from normal parenchyma. In what seemed to be the earliest stage there was no infiltration

of wandering cells into or about the area (Fig. 7). The next stage was the presence of intense polymorphonuclear cell infiltration at the periphery (Fig. 9), then advance of these cells into the necrotic area with fragmentation and disappearance of liver cells (Fig. 10). In some areas this advance reached the center of the area and there was often also destruction and nuclear fragmentation of the invading cells. Occasionally an area contained a central zone of complete disintegration of all cells. What appeared to be the latest stage encountered consisted of mononuclear and multinuclear cells at the periphery (Fig. 11), and proliferation of parenchymatous cells in this region, while toward the center the phagocytosis of necrotic liver cells was still progressing. No evidence of scar formation resulting from this process was seen even in the infections of longest duration, and relatively early stages of the necrotic process were found in an infection of 65 weeks duration.

The earliest occurrence of coagulative necrosis in a unisexual infection was found at 2½ weeks. From this time onward up to 70 weeks 11 per cent of the male infections and 24 per cent of the female infections had areas of necrosis. In the bisexual infections the earliest infections in which coagulative necrosis was found were of 2 weeks duration and from that time through 5 weeks it was found in 40 per cent of the infections. After the first appearance of these lesions the frequency of their occurrence seemed to have no relation to the duration of the infection.

Connective tissue stain. Sections from a few livers at various intervals after infection were stained for connective tissue. In none of the unisexual infections even up to 70 weeks duration was there evidence of more than delicate connective tissue fibrils in the perivascular or parenchymal cellular infiltrations or at the periphery of older necrotic areas. In one bisexual infection of 5 weeks duration there was a definite increase of larger connective tissue fibrils extending out into the parenchyma from some of the areas of periportal infiltration.

Worms in portal venules. Schistosome worms were seen in portal venules in sections from some of the infections of 3½ weeks or longer. As stated above, their presence was sometimes associated with areas of coagulative necrosis immediately adjacent to or near the venule (Figs. 7 and 8), but usually there was no such association. Although periportal cellular infiltration was sometimes present adjacent to the worms, in other cases there was none. No damage to the intima of the vessel wall adjacent to the worms was apparent. Thrombosis of portal venules was not found in any of the unisexual infections. It was seen in 6 of the bisexual infections but was not associated with the presence of worms. These thrombi were all small and did not occlude the lumen of the vessel.

Haematin pigment. Haematin from blood digested in the ceca of the schistosome worms and deposited in the Kupffer cells and mononuclear phagocytes was found in increasing amounts as the duration of the infections increased. It was more abundant in male than in female infections, probably because the male worms, being larger, ingest more blood. In the late infections it was also deposited in large masses outside of demonstrable cells in the portal spaces and along blood capillaries in the parenchyma (Fig. 4).

Unisexual and early bisexual infections with S. japonicum. Owing to the difficulty of obtaining infected *Oncomelania* snails we have obtained histological sections of mouse livers from only 29 male infections and 22 female infections. The male infections have ranged from 2 to 95 weeks in duration, the female infections from 3 to 70 weeks in duration. Periportal and parenchymal cellular infiltration was found in a smaller proportion of animals, and generally was less intense than in *S. mansoni* infections, and the infiltrations were mainly of mononuclear cells. In only 2 mice, both female infections of 12 and 16 weeks duration respectively, were areas of coagulative necrosis of the parenchyma seen. These areas were very small, with a few necrotic liver cells and intense polymorphonuclear cell invasion. They resembled the lesion described in one old uninfected mouse more than the large, sharply demarcated necrotic areas in infected *mansoni* mice.

Of the 4 mice having bisexual *S. japonicum* infections of 2 to 5 weeks duration only 2 showed pathological lesions. In a 2-weeks infection the liver parenchyma contained many areas of cellular infiltration, and in addition several large areas of coagulative necrosis. In a 4-weeks infection there was considerable periportal and parenchymal cellular infiltration but no necrosis. In this mouse a few *S. japonicum* eggs were seen in portal venules without any surrounding cellular reaction.

Unisexual infections with S. haematobium. Only 17 male infections have been available for study. They ranged from 2 to 65 weeks in duration. Since mice are not very suitable hosts for *S. haematobium* the number of worms recovered at autopsy varied greatly and was never very large. No worms were recovered from 6 of these mice, although one, infected 65 weeks previously, had heavy pigment deposits in Kupffer cells, indicating that worms had been present. Worm recovery from the other mice ranged from 1 to 22, which is much less than the average from *S. mansoni* and *S. japonicum* mice, although the number of cercariae employed was usually larger. In 3 infections there was moderate periportal cellular infiltration and in 2 it was intense. In only 1 was there intense parenchymal infiltration. Two of the infections showed areas of coagulative necrosis in the parenchyma. In one of these, a 6-weeks infection, the lesion was very small with considerable polymorphonuclear cell infiltration; in the other, a 55-weeks infection, the lesion was large, nearly encircling a portal space but not directly adjacent to it and having very little cellular infiltration at the periphery.

DISCUSSION

These observations add to the evidence that pathological changes in the liver are sometimes associated with the presence of either male or female schistosome worms or both in the mesenteric-portal blood stream, independent of the deposition of eggs. The inconstancy of occurrence of these changes in mice, their wide variation in intensity and their frequent lack of direct contact with worms make it difficult to be certain of their origin, but the general trend of increase in severity in proportion to duration of infection up to 2 to 3 months in unisexual infections, strongly favors the view that they are caused by a toxic product of

the worms. The periportal infiltration, starting as a small mononuclear cell reaction, may become predominantly polymorphonuclear, but after about 4 months tends to return to mononuclear. The parenchymal infiltration is much more likely to be predominantly polymorphonuclear from the beginning, and usually has entirely disappeared after the fourth month. This suggests that the polymorphonuclear reaction is an allergic phenomenon which, like the early symptoms and eosinophilia in man, tends to disappear later in the course of the infection. In bisexual infections the cellular infiltration persists after the lesions produced by eggs begin to develop. The two types of lesions merge, and this diverts attention to the egg lesions and leads to the tendency to attribute the entire pathology to the eggs.

The areas of coagulative necrosis which we have described here, and which seem to be similar to those found by Jaffé, Mayer and Pifano (1945), are different from those described by Fairley (1920) in hyper-infected monkeys or by Faust and Meleney (1924) in a hyper-infected dog. They have the appearance of infarcts. The only difference from infarcts as they are seen in such organs as the kidney, spleen, and lung is that the liver lesions appear to leave no permanent scar, probably due to the ability of the parenchymal cells to regenerate. If they are infarcts caused by obstruction of portal venules it is logical to assume that worms are the obstructing agents. This assumption is supported by the presence of worms in venules adjacent to some of the lesions. Since the worms move about, and do not usually damage the vessel wall, it is conceivable that they obstruct the circulation only long enough to produce necrosis of an area of parenchymatous cells and then withdraw the obstruction. They may even withdraw from their original position during fixation of the tissue for sectioning.

It is interesting that these areas of necrosis, as well as the periportal and parenchymal cellular infiltrations, were more marked in bisexual than in unisexual infections of similar duration up to 5 weeks. This suggests that the presence of both sexes may have a more toxic effect on the liver, and that the more rapidly growing worms more often obstruct the portal venules.

Since our material from *S. japonicum* and *S. haematobium* infections was not as abundant as that from *S. mansoni* infections, it is impossible to compare accurately the relative intensity of the liver lesions associated with the three different species of worms. Comparison is also difficult because fewer *S. haematobium* worms reach the portal-mesenteric vessels in mice, and they develop more slowly. For these reasons our impression that the average intensity of the lesions in *S. mansoni* infections was somewhat greater than that in *S. japonicum* or *S. haematobium* infections may be erroneous.

The absence of fibrosis in our unisexual infections, even as long as 95 weeks duration, suggests that, in mice at least, something more than a single sex of the worms is required to produce cirrhosis of the liver. This agrees with the observations of Lee (1932) in hamsters. It is contrary to the hypothesis of Girges (1934) that the cirrhosis of the liver in Egyptian splenomegaly is caused by unisexual male infections with *S. mansoni*. It may be hazardous to use mice as a criterion for what may occur in man over a much longer period of time. However, rats have been shown to develop cirrhosis of the liver following ne-

crosis caused by dietary deficiencies (Himsworth, 1950), and mice develop the typical fibrous scars of schistosomiasis following egg deposition. One possible explanation of the "pipestem cirrhosis" in longstanding infection with *S. mansoni* in man, where few eggs are demonstrable in the liver, is that a few worms of both sexes are present, the pathological process is initiated by toxins from the worms and from eggs deposited in the liver, and the final severe cirrhosis is a combined product of the infection and of dietary deficiency.

SUMMARY AND CONCLUSION

1. Histological sections of livers from white mice infected with male or female *Schistosoma mansoni*, *S. japonicum* or *S. haematobium*, and with both sexes of *S. mansoni* or *S. japonicum* prior to egg deposition, were examined to study lesions associated with the presence of worms in the portal-mesenteric vessels in the absence of lesions produced by eggs. Most of this material came from *S. mansoni* infections.

2. In unisexual *S. mansoni* infections periportal infiltration of mononuclear and polymorphonuclear leucocytes appeared after 3 to 4 weeks in the majority of unisexual infections and increased in intensity in infections up to about 12 weeks in duration, after which it tended to become less intense and was predominantly mononuclear.

3. Cellular infiltration in the parenchyma of the liver, predominantly polymorphonuclear, occurred in some animals, apparently independent of extensions from the periportal infiltrations. These increased in frequency and intensity in infections up to about 12 weeks in duration, but were rare in infections 20 weeks or more in duration.

4. Areas of coagulative necrosis in the parenchyma of the liver were found in a considerable number of animals. They became invaded by polymorphonuclear leucocytes with later disappearance of liver cells and invasion of mononuclear and multinuclear cells. Worms were sometimes present in venules immediately adjacent to these lesions, giving the impression that they may have been infarcts initiated by temporary obstruction of the blood supply by the worms.

5. No evidence of development of cirrhosis of the liver was found in unisexual infections up to 95 weeks in duration.

6. Some of the unisexual infections with *S. japonicum* or *S. haematobium* showed the same types of lesions as those infected with *S. mansoni*. In general the lesions were less intense, but accurate comparison is not warranted.

7. In bisexual *S. mansoni* infections up to 5 weeks in duration periportal and parenchymal cellular infiltration and coagulative necrosis appeared earlier and were more frequent and intense than in unisexual infections of the same duration. Occasional mural thrombi were found in portal venules.

8. Some uninfected mice had mild degrees of periportal and parenchymal cellular infiltration, but areas of coagulative necrosis were not seen.

9. Although some infected mice failed to show any lesions in the liver, it seems possible that the intense cellular infiltrations and areas of necrosis were causally related to the presence of worms in the mesenteric-portal blood vessels.

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PLATE I

FIG. 1. Liver of an uninfected young adult mouse showing minimal periportal and parenchymal infiltration of mononuclear and polymorphonuclear cells. ×200.

FIG. 2. Liver of an uninfected mouse one year old showing a single large area of periportal and parenchymal infiltration of mononuclear and polymorphonuclear cells with destruction of parenchymatous liver cells. ×200.

PLATE I

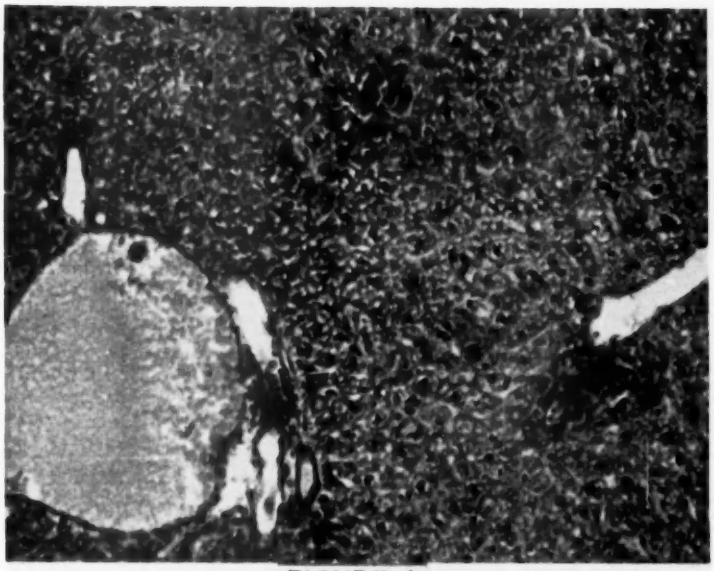

FIGURE 1

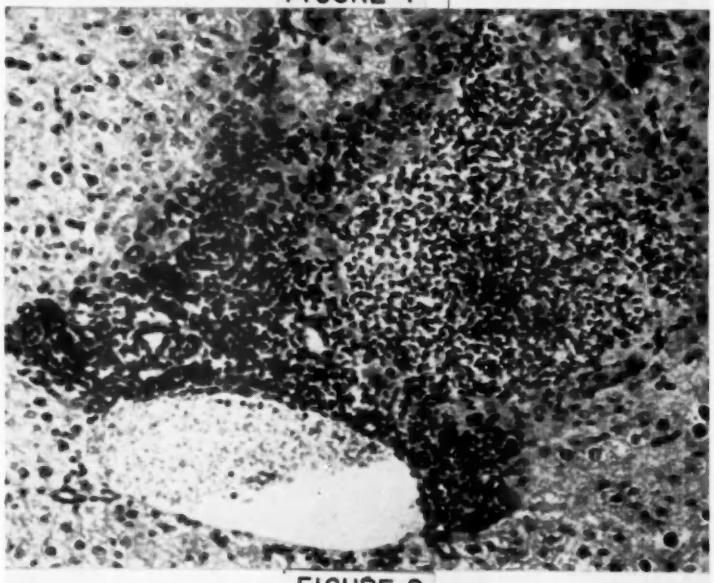

FIGURE 2

PLATE II

FIG. 3. Liver of mouse with unisexual female *S. mansoni* infection, duration 4 weeks. Intense periportal and parenchymal infiltration, polymorphonuclear cells predominating. ×200.

FIG. 4. Liver of mouse with unisexual male *S. mansoni* infection, duration 25 weeks. Intense periportal mononuclear infiltration and large deposits of haematin pigment. ×200.

PLATE II

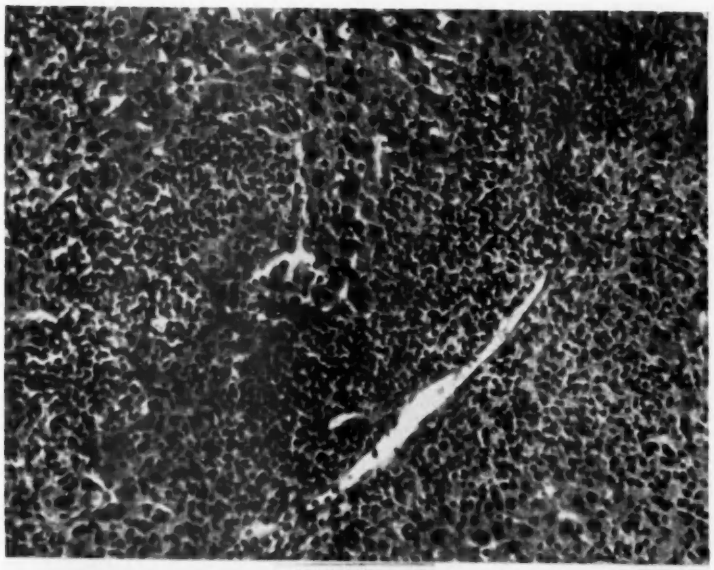

FIGURE 3

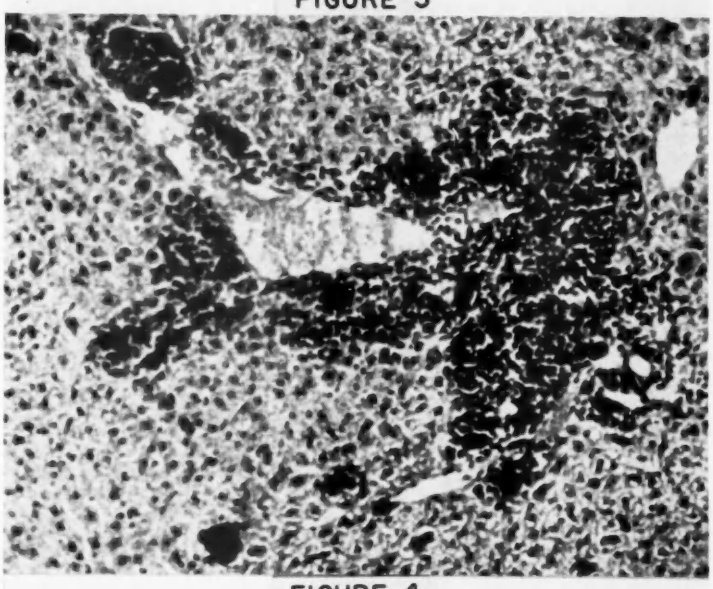

FIGURE 4

PLATE III

FIG. 5. Liver of mouse with unisexual female *S. mansoni* infection, duration 16 weeks. Intense periportal mononuclear infiltration and a few small deposits of haematin pigment. ×140.

FIG. 6. Liver of mouse with bisexual *S. mansoni* infection, duration 4 weeks. Intense periportal and parenchymal infiltration, polymorphonuclear cells predominating. ×150.

PLATE III

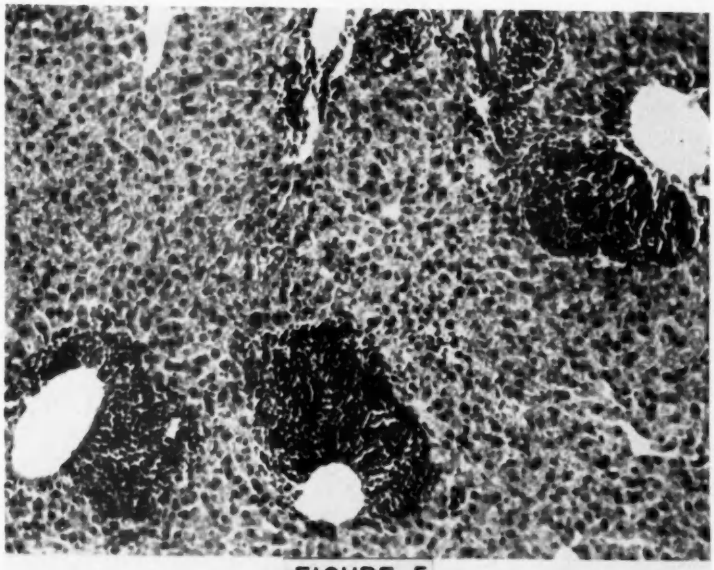

FIGURE 5

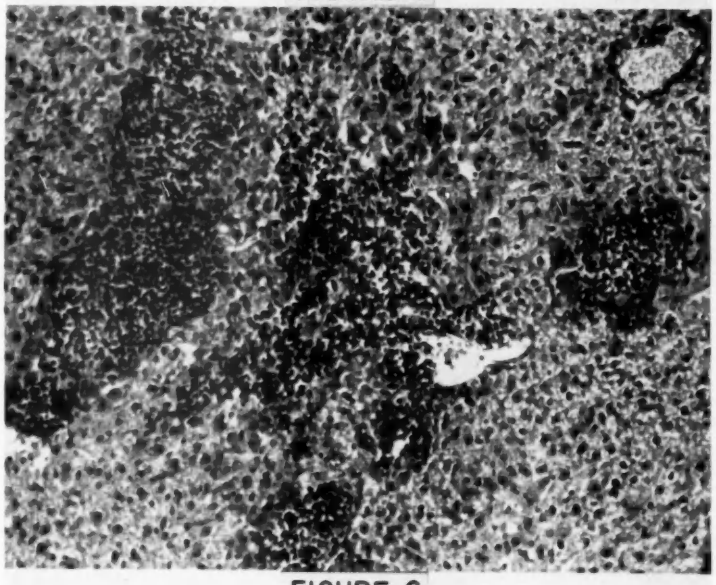

FIGURE 6

PLATE IV

FIG. 7. Liver of mouse with unisexual female *S. mansoni* infection, duration 6 weeks. Coagulative necrosis of parenchyma sharply demarcated from normal tissue and adjacent to portal venule containing worms. Note slight periportal infiltration. In the necrotic area nuclei of parenchymatous cells are shrunken and pyknotic. ×150.

FIG. 8. Liver of mouse with unisexual female *S. mansoni* infection, duration 6 weeks. Two areas of coagulative necrosis near a portal venule obstructed by a worm. Note beginning infiltration of polymorphonuclear cells at periphery of necrotic areas. ×150.

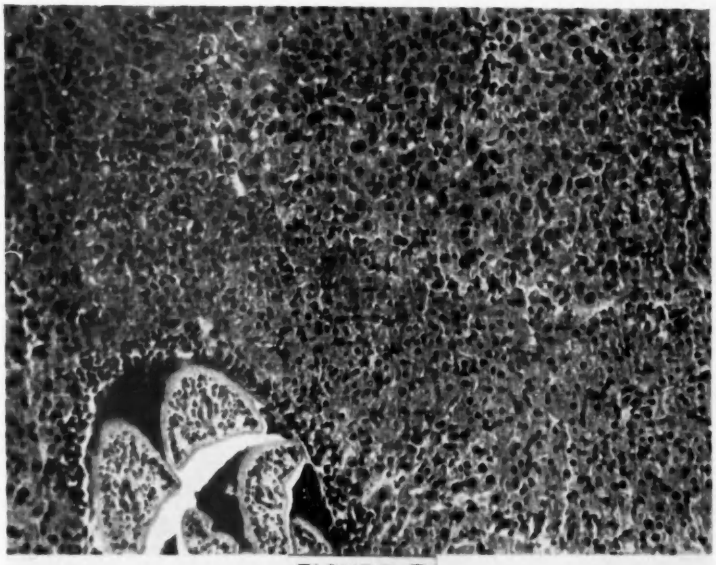

FIGURE 7

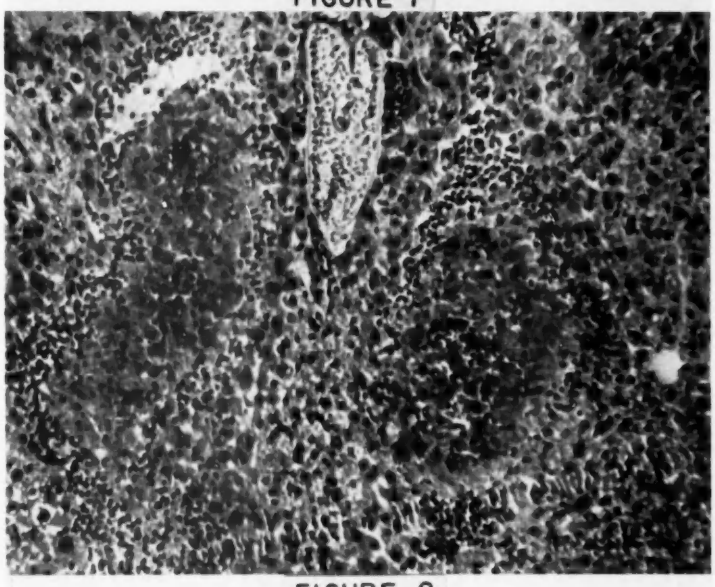

FIGURE 8

PLATE V

FIG. 9. Liver of mouse with unisexual male *S. mansoni* infection, duration 3½ weeks. Area of coagulative necrosis with intense peripheral polymorphonuclear infiltration and partial disappearance of necrotic parenchymatous cells. ×150.

FIG. 10. Liver of mouse with unisexual female *S. mansoni* infection, duration 5½ weeks. Large area of coagulative necrosis involving a portal venule. Polymorphonuclear infiltration in the necrotic area at its periphery and in surrounding parenchymatous tissue. ×140.

PLATE V

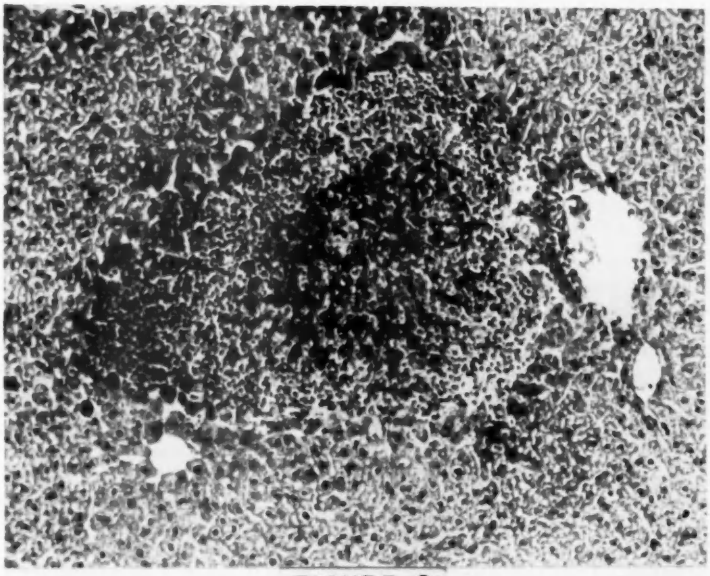

FIGURE 9

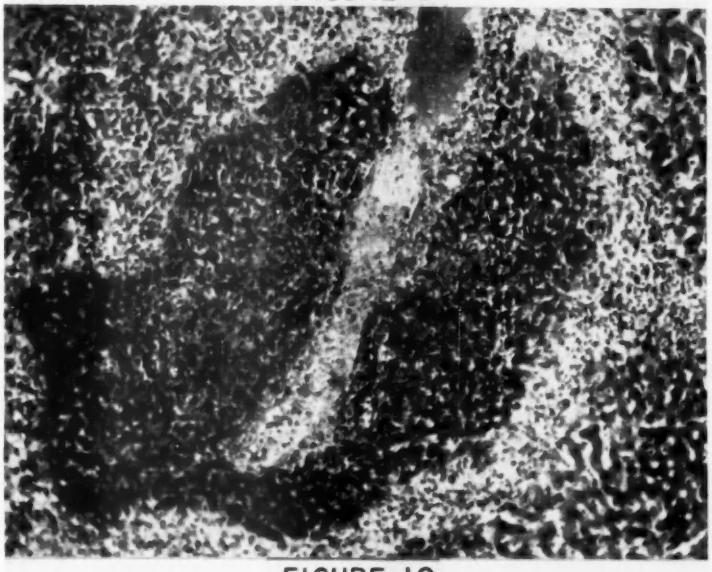

FIGURE 10

PLATE VI

FIG. 11. Liver of same mouse as Figure 10. Multinuclear, mononuclear, and polymorphonuclear cells in a delicate connective tissue reticulum adjacent to an area of coagulative necrosis. No evidence of development of a fibrous scar. ×150.

PLATE VI

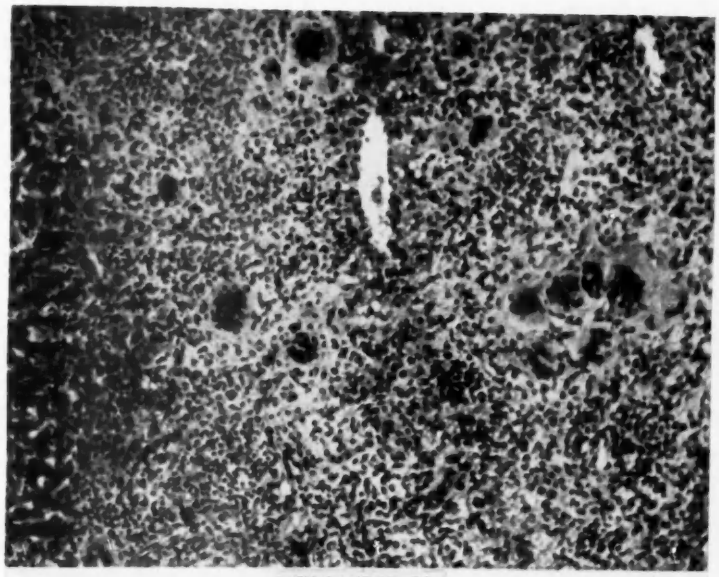

FIGURE II

STUDIES ON SCHISTOSOMIASIS JAPONICA IN FORMOSA

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PART I. HISTORIC REVIEW OF THE EPIDEMIOLOGY OF SCHISTOSOMIASIS JAPONICA IN FORMOSA

Schistosomiasis japonica prevails in Japan, China (including Formosa), the Philippines, and Celebes. In literature of importance on tropic diseases and parasitology, however, only a very meager reference to this disease in Formosa is found. Faust and Meleney (1924) state, "In Formosa an endemic center of schistosomiasis has been located by Yokogawa (1915) in the vicinity of Shinchiku, near the center of the island, where man, cattle, oxen, dogs and hogs are said to be infected. Specimens of cercariae and adults, which Doctor Yokogawa has been kind enough to send to us, are morphologically identical with *Schistosoma japonicum*." On the other hand, Craig and Faust (1951) mention, "In Formosa there is an enzoötic region near the northwest part of the island". Manson-Bahr (1945), Strong (1944), and Simmons, *et al.* (1944) write that schistosomiasis is prevalent in the southern part of Formosa. Mao (1948), however, lists only Shinchiku in the north-western part of this island as the endemic area.

Concerning the intermediate host, *Oncomelania formosana*, Manson-Bahr (1945) specifies that it occurs in South Formosa, whereas Wright (1947), in accordance with the reports of the other investigators, lists Shinchiku in his distribution record. Kuroda (1941) and Abbott (1948) state that this species has been found at Rono, Kosyu, Kansai, and Sintiku (Shinchiku).[1]

From the above cited literature, it can be safely inferred that schistosomiasis japonica does occur in Formosa and that its intermediate host is *O. formosana*. However, concerning the endemic area of the disease as well as the occurrence of the snail, there is no unanimous record as to whether it is found in North Formosa, Central Formosa and South Formosa. In order to settle these controversial points, and to review the work which had been done in the past as a basis for our investigation, we deem it necessary to present, from the Japanese literature, an historical review of the epidemiology of schistosomiasis japonica in Formosa.

ORTHOGRAPHY OF FORMOSAN PLACE NAMES

After World War II, the orthography of Formosan place names, formerly in Japanese, is now in Chinese. To avoid confusion, it is necessary to give the Chinese equivalents of all the Japanese locality-names mentioned in the introduction. In the other parts of this paper, only the Chinese names will be used.

(1) *Kansai:* called Kuanhsi in Chinese, is a village in Hsinchu *Hsien* (Schinchiku Prefecture); (2) *Kosyu:* called Haohsiu in Chinese, is a village in Changhua *Hsien* (Taichu Prefecture) and located in the mid-western part of Formosa;

[1] We are informed by Dr. Abbott that the source of his information is Kuroda (1941).

(3) *Rono:* called Laonung in Chinese, is a village in Kaohsiung *Hsien* (Takao Prefecture) and located in the south-western part of Formosa; and (4) *Shinchiku* or *Sintiku:* called Hsinchu *Hsien* in Chinese, is located in the north-western part of Formosa.

FIG. 1. Island of Formosa, showing the known distribution of *Oncomelania formosana* as appeared in the Japanese literature. The villages where living specimens of *O. formosana* have been found are indicated by a snail in total black and the village where semifossil specimens have been reported by a snail in white. Japanese equivalence of the locality-names in this map: (1) Changhua-Shoka, in the former Taichu Prefecture; (2) Haohsiu-Kosyu; (3) Hiaopuhsin-Shohoshin; (4) Hsinchu-Shinchiku or Sintiku; (5) Kaohsiung-Takao; Kuanhsi-Kansai; and Laonung-Rono.

REVIEW OF THE EPIDEMIOLOGY OF SCHISTOSOMIASIS JAPONICA IN FORMOSA FROM JAPANESE LITERATURE

(Figs. 1–2)

In Formosa, *S. japonicum* was first discovered in 1914 by Takegami (1914) in the portal vein of a pig from Lienchiaotso (a village in the southern part of

Changhua *Hsien*). Later on, he found that this trematode was present also in dogs, goats and oxen of the above-mentioned village. Examination extended to a number of neighboring villages revealed that specimens of this worm were found in pigs, dogs, goats, oxen and buffaloes in 17 additional villages in this area: Luchiang, Kuanyutso, Chenping, Wayao, Putzu, Niuputso, Puyen, Hsishihtso, Paitou, Lukoutso, Hsiaopuhsin, Louyao, Pehtou, Pehshihliao, Chitzuting, Sanshihchangli, and Yuanlin (Takegami, 1914, 1915; Takegami *et al.* 1914). Takegami estimated that in the two villages, Lienchiaotso and Hsiaopuhsin, more than 70 per cent of the pigs were positive for this parasite.

Takegami also examined in this area urine and stools of patients for the ova of *S. japonicum* and found that two stools from Hsiaopuhsin and one from Luntzu were positive for the ova of this parasite. On the other hand, Yoshida and Kubo (see Yokogawa, 1915) and Inabe (see Iwata, 1940) failed to find any human cases of this disease in the same area. In 1940, Iwata (1940) stated that up to the time of his writing, no human case of schistosomiasis had ever been reported in Formosa. Evidently Takegami's identification of the eggs of *S. japonicum* was not accepted beyond question by his contemporary and later Japanese colleagues.

Concerning the intermediate host of this parasite in Formosa, Yokogawa (1915) obtained, in the irrigation ditches at Hsiaopuhsin, snails which were identified as *O. formosana* (Yokogawa, 1916). However, this was not the first report of its presence in Formosa. As early as 1905, Pilsbry and Hirase (1905) recorded its finding at Laonung (=Rono) in Kaohsiung *Hsien*. But its incrimination as the intermediate host of *S. japonicum* was not known before 1915. Yokogawa got from the snails from Hsiaopuhsin cercariae which were proved to be the cercariae of *S. japonicum* by the morphological characters of the adult worms obtained from an experimentally infected rabbit. The specific identification of Yokogawa's specimens was confirmed by Faust and Meleney (1924).

Subsequently, Horikawa (1935) reported his finding of *O. formosana* at Haohsiu (=Kosyu) in Changhua *Hsien*. In 1941, Kuroda (1941) reported the presence of a semifossil specimen of *O. formosana* at Kuanhsi (=Kansai) in Hsinchu *Hsien* (=Shinchiku). However, except in Hsiaopuhsin, no examination of the snails for cercariae was carried out.

DISCUSSION AND SUMMARY

The historical review of the epidemiology of schistosomiasis japonica in Formosa from Japanese literature as given above shows clearly that the chief endemic area of this disease is Changhua *Hsien* (Taichu Prefecture) in the mid-western part of this island. Possibly another endemic area exists at Laonung in Kaohsiung *Hsien* (Takao Prefecture), in the south-western part of Formosa. The current idea abroad that Shinchiku (=Hsinchu *Hsien*) in the northwestern part of the island is the main endemic area of this disease is without foundation in the Japanese literature. Dr. S. Yokogawa, in a personal communication, dated March 25, 1951, agrees with us and states, "It is true that there was no schistosomiasis japonica in Shinchiku, either human case or animal case."

There is, furthermore, no report in the literature of the finding of living *Oncomelania* snails in Shinchiku.

The above review also shows that the riddle of the human cases of schistoso-

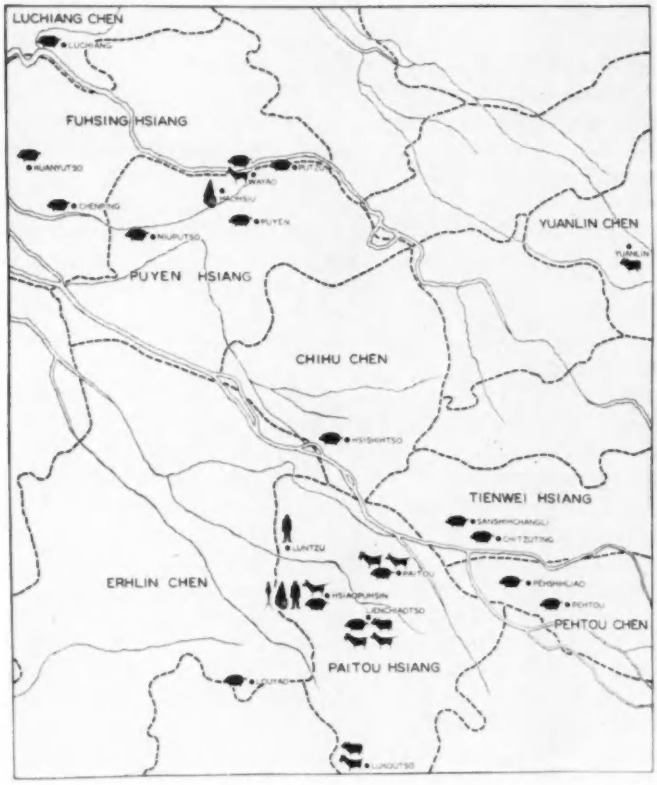

FIG. 2. Symbolic representation of the epidemiology of schistosomiasis japonica in the endemic areas in Changhua *Hsien* as appeared in the Japanese literature:

"*Man*", "*Pig*", "*Cattle*", "*Goat*", or "*Dog*", indicates villages where adult worms or eggs of *S. japonicum* have been reported from the various final hosts;

"*Snail*" indicates the villages where *O. formosana* has been found; and

"*Cercaria*" indicates the village where cercariae of *S. japonicum* have been reported in *O. formosana*.

miasis as given by Takegami should be solved, the incidence of animal infection verified, and the distribution of the intermediate host, *O. formosana*, in this island thoroughly re-studied.

Part II. On the Habits, Distribution and Incidence of Infection of Oncomelania Formosana, in Changhua Hsien, Formosa[2]

As the chief endemic area of schistosomiasis japonica in Formosa is in Changhua *Hsien*, in the mid-western part of the island, we decided to start our epidemiological study of this disease in this county. Our original intention was first to find out the possible presence of human cases of schistosomiasis in the endemic area in order to solve Takegami's riddle of "three human cases of schistosomiasis." Owing to circumstances beyond our control, we modified our plan and began with the investigation of the habits, distribution, and incidence of infection of the snail intermediate host, *O. formosana*, in this area.

Our historical review of the epidemiology of this disease indicates that in Changhua *Hsien*, *O. formosana* has been reported only from two villages, Hsiaopuhsin and Haohsiu, and cercariae of *S. japonicum* were found only in the snails collected from Hsiaopuhsin. Other data on *O. formosana* in this area are completely lacking. In the following pages, the results of our studies on the habits, distribution and incidence of *O. formosana* are presented.

TOPOGRAPHICAL AND METEOROLOGICAL DESCRIPTION OF CHANGHUA HSIEN

Changhua *Hsien* (Figs. 1, and 5) is situated in the center of Formosa on the western seaboard and is therefore in the vicinity of the Tropic of Cancer. Its shape is like an equilateral triangle with its apex directed northward and its base towards the south along the Hsilu River. Between its eastern border which is bounded by the Paku Hills and the Strait of Taiwan on its western side, lies a rich fertile plain devoted to the cultivation of rice. This area is irrigated by the Hsilu River and its main tributary, the Choshui River. The water is distributed by a well planned system of irrigation canals which are controlled by sluices and locks.

The whole district has an area of 1,061 sq. kms., about 920 sq. kms. of which are under cultivation. Geologically, this district belongs to the quaternary period with alluvial and diluvial deposits. The former covers the most important agricultural land, while the latter forms the elevated stratum on the eastern border of the county. Meteorologically this district has a subtropical climate. The mean monthly temperature ranges from 15.7°C. to 27.7°C. The yearly variations are between 0.4°C and 39.3°C. The average annual rainfall is 1,199 mm. These climatic conditions favor the production of two crops of rice a year.

RESULTS OF THE INVESTIGATION

I. Habits of O. formosana *in Changhua Hsien*

Before we report the results of our studies on the habits of *O. formosana*, it seems pertinent to describe here the irrigation system of this area. As mentioned above, practically the whole plain is irrigated by the water from the Hsilu

[2] The expense of the field work for the study as given in this part was subsidized by the Joint Commission on Rural Reconstruction, Taipeh.

River and its tributary, the Choshui River. The water in these rivers as well as in the irrigation ditches originating from them is very turbid, because a large portion of the drainage basin of the Hsilu River is made up of slate formation. We may arbitrarily classify the irrigation ditches in this area, according to their width, into four kinds. Ditches of the first order are those of about 5–20 meters, averaging about 12 meters, in width, and the water is led directly from the Hsilu River or the Choshui River. Ditches of the second order are about 2–5 meters wide with the average width of about 3.5 meters. They are primary divisions of channels of the first order. Ditches of the third order are about 1–2 meters (average 1.4 meters) in width and are leads-off from the second order. Ditches of the fourth order (Figs. 3 and 4) are about 0.25–1.0 meter (average

FIG. 3. An irrigation ditch of the fourth order at Sansheng, Puyen *Hsiang*, showing the width of the ditch, depth of water, and vegetation therein. Dr. H. F. Hsü was searching for *Oncomelania* snails in the ditch.

0.5 meter) in width, are branches of the ditches of the third order and form the irrigation channels between the paddy fields.

The snails, *O. formosana*, are usually found in the ditches of the fourth order, occasionally in the ditches of the third order, rarely in the ditches of the second or the first order. Even in the ditches of the fourth order, the snails are not ordinarily found in the ditches between the paddy fields.

In this area, farm houses are generally surrounded by bamboo groves, especially those which are situated on the outskirts of a village. Paddy fields are usually located immediately outside the village. Under such a bamboo grove there is an irrigation ditch of the fourth order and, very often, it is an end-ditch (Fig. 4). Water in these ditches is comparatively more stagnant and their soil has more

organic matter than others. As this area is an open country near the seaboard, the strong northeast and southwest monsoons blow unhampered. Accordingly rows of bamboos are sometimes planted along one side of the irrigation ditches of the fourth order for the protection of the rice plants from the damage of strong winds. Such ditches are therefore well shaded by bamboo groves and also rich in organic matter or humus which results from the accumulation of the decaying fallen leaves. It is in these humus-laden ditches that specimens of *O. formosana* are generally found.

Occasionally *Oncomelania* snails are found in irrigation ditches of the third order which are likewise shaded by bamboo groves. As water in these ditches

FIG. 4. Tayu, Puyen *Hsiang*, showing a typical breeding place for *O. formosana*. Dr. H. F. Hsü and Mr. Chen Chin-cheng were making collection of the *Oncomelania* snails in an end-irrigation ditch of the fourth order. The ditch is situated just on the outskirts of a farm and is shaded by a bamboo grove.

usually runs faster, and the organic matter in the soil is also less than in the ditches of the last order, *Oncomelania* snails are also less frequently found. In the irrigation ditches of the second or the first order, *Oncomelania* snails are practically absent. However, on two occasions and only for a short stretch, we found some *Oncomelania* snails in ditches of such width, once on the bank of an irrigation ditch 11 meters wide and another time on the bank of an irrigation ditch 13 meters in width.

One remarkable condition in relation to the habits of *O. formosana* in this endemic area should be specially pointed out, namely that *Oncomelania* snails thrive in water turbid with fine suspensions of silt and clay particles.

The other details of the habits of *O. formosana* are similar to those described

for other *Oncomelania* species in other regions of the Orient, viz., it is amphibious, living within the range of about 20 cm., mostly 10 cm., above or below the water level on the banks of the ditches. It is very seldom seen in the rice fields.

FIG. 5. Changhua *Hsien*, showing the distribution of villages harboring *Oncomelania* snails in this county. All the villages where *Oncomelania* snails have been found are indicated in this map by black dots: one dot representing one *Oncomelania*-snail-harboring village. The rectangle represents the area enlarged in Fig. 6.

II. *Distribution of* O. formosana *in Changhua Hsien*

In this district, we have searched practically all irrigation ditches which were likely to harbor the *Oncomelania* snails. Our survey was carried out on foot throughout the whole plain from December, 1949 to August, 1950. Before we recorded a negative finding, all the ditches in and outside the village were carefully gone over. As our purpose is only to find out the extent of the distribution of the snails in this district, we usually left one village for another after finding the *Oncomelania* snails. In other words, we did not try to find out the total number of positive ditches unless there was some special reason.

As mentioned above, the whole county covers an area of 1,061 sq. kms., of which 273 sq. kms., are positive for *O. formosana*, i.e., about one-fourth of the whole county (Fig. 5). The positive area is quite wide, covering about one third of the rice growing district. Most of the positive villages are located in the central part, especially those situated north of the Choshui River. The northernmost portion, the eastern hilly regions, the southeastern corner and a very narrow zone along the western seaboard are, however, free from this molluscan species.

Changhua *Hsien* is administratively subdivided into 26 townships. In nineteen townships, *O. formosana* are present. They are: Changhua *Shih*, Fuhsing *Hsiang*, Huatan *Hsiang*, Puyen *Hsiang*, Shetou *Hsiang*, Tienwei *Hsiang*, Chichow *Hsiang*, Erhlin *Chen*, Tacheng *Hsiang*, Luchiang *Chen*, Tatsung *Hsiang*, Chihu *Chen*, Yuanlin *Chen*, Yungtsing *Hsiang*, Paitou *Hsiang*, and Chutang *Hsiang* (Fig. 6). In Puyen *Hsiang*, *Oncomelania* snails are found practically in every village and it is, therefore, the most important township for the *Oncomelania* snails in this county.

Altogether 628 villages[3] in this county were surveyed for *Oncomelania* snails. Amongst them, *O. formosana* is present in 140 villages. The names of the positive villages for this mollusc are given under their respective townships in the following section (see also Fig. 6).

III. *Incidence of* O. formosana *Infected with the Cercariae of* S. japonicum *in Changhua Hsien*

In order to add useful knowledge to the epidemiology of schistosomiasi[8] japonica in this county, the occurrence of infected *Oncomelania* snails in different *Oncomelania*-positive ditches and villages in this region was investigated.

Procedures and Methods. For such a study, it is of course much better to examine as many snails as possible in one irrigation ditch and at the same time to examine as many irrigation ditches as possible in *Oncomelania*-positive villages. As our time and technical personnel were very limited, and as it is, furthermore, very time-consuming to collect and examine a large number of snails, we decided arbitrarily to study only 300 *Oncomelania* snails from one ditch in each village. Owing to the paucity of the snail population in some villages, the intended number of snails for examination, 300 from one ditch, was not obtained in all ditches. Altogether 38,241 snails from 140 ditches of 140 positive villages were investigated, making an average of 273 snails per ditch. We are certain that the number of villages with positive finding of cercariae in *Oncomelania* snails in this county would be more than the 75 given below, if more snails from a ditch and snails from a larger number of ditches could have been examined.

The examination of *Oncomelania* snails for cercariae was done by crushing living snails between two slides, three snails being crushed at the same time.

[3] The names of the villages used in this paper are after those given in the 1/25,000 military map published in 1926 by the Land Measurement Bureau, Ministry of the Army, the Imperial Japanese Government and reprinted by the Ministry of National Defence, Chinese Government, 1949.

One drop of water was added to a crushed specimen and the latter was teased and examined under a binocular dissecting microscope. Finding of sporocysts

FIG. 6. Endemic area of *O. formosana* in Changhua *Hsien*, shown enlarged. Villages where infected *Oncomelania* snails were found are indicated by black dots, while villages where *Oncomelania* snails have been found but *Schistosoma* cercariae have not been detected in the snails are represented by empty circles.

alone was also regarded as a positive infection. All the examinations were done from June to August, 1950.

Results. The names of the villages where the *Oncomelania* snails were found, and the percentage of the snails positive for cercariae (in parenthesis) in each ditch in each village are given in the following list (See also Fig. 6):

CHANGHUA SHIH: Nanwei (0).

LUCHIANG CHEN: Pehtou (0), Luntzuting (0.7), Chuyuantzu (0).

FUHSING HSIANG: Chiaotou (0), Fuhsing (2), Fouching (0), Fanshe (0), Hsialiao (1), Hsiushuitso (0.7), Nientso (0), Kuanyutso (2.8), Hungchuehliao (0.7), Maiyutso (0.7), Waichushuikou (0), Chenping (2), Ali (2), Waihouchi (1.3).

HSIUSHUI HSIANG: Hsiushui (0), Chiangkantso (0), Hsinhsing (0), Tsengtsotso (2.3), Chinhsunyang (0), Shanhsi (0).

HUATAN HSIANG: Kouchuang (0.7), Luntzutou (0), Huatan (2), Liutso (0.3), Chintun (0).

TATSUN HSIANG: Chiatunglin (0), Tachiaotou (0), Tingchiotzu (0), Lienhuachih (0.3), Tatsun (0), Kuokou (0), Chiangwei (4), Tahu (0.7), Paitang (0), Kunchi (0.3), Nanshihtzu (0.3), Shuichueh (2.5), Pehshihtzu (0), Tienwei (0).

PUYEN HSIANG: Paoan (1), Hsinhsing (0.7), Fantungpu (0.7), Tayu (3.7), Wayao (3), Putzu (0), Chushuikou (1.7), Haohsiu (7.3), Wailuntzuchie (5.1), Hsishihhu (1.3), Puyen (3.7), Nanchiang (0), Tsaitang (3), Talien (0.7), Luntzuchie (0), Chioshuchie (0), Shangnanshihpu (0.7), Hsiananshihpu (0.3), Niuputso (0.7), Puhsintzu (0.3), Shihpaichie (0.7), Chinshui (1), Shihchiuchien (2.2), Hsinchuangtzu (0), Sansheng (3).

CHIHU CHEN: Shanliao (0), Tingliao (0.3), Chuweitzu (0), Tinsankuaitso (0), Pehshihwei (30), Waiszukuaitso (0), Chutien (1.3), Toumentou (9), Luntzuchiehsia (6.3), Fanpo (2), Sankuaitso (0), Sanchiotzu (0.3), Hsishihtso (0, 3), Chutoutzu (1.3), Tsaopu (0).

PUHSIN HSIANG: Wufeng (2), Erhchungnan (0), Chiukuan (2), Takouwei (0), Hsinkuan (0), Lutso (1.1).

YUANLIN CHEN: Taputso (0), Nanping (0), Tasanchiotan (0.3), Tajao (0), Chientsotso (0).

SHETOU HSIANG: Pinglang (0), Chiutsotso (0).

YUNGTSING HSIANG: Nukan (0.7), Hulien (0.3), Tunganchai (0), Szukuaitso (0.3), Yungching (0), Tuao (0), Chutzuchie (0), Luntzuwei (2.7), Nanchianghsi (0), Nanchiangchiu (0.3).

TIENWEI HSIANG: Sanshihchangli (0), Hsiatienwei (0), Liushunan (0), Talien (0), Chuluwei (0), Hsintsotzu (1), Chenping (0), Hsiaohungmaoshe (0.3).

PAITOU HSIANG: Hsiaopuhsin (0), Sankuaitso (0).

CHICHOW HSIANG: Shuiwei (0).

CHUTANG HSIANG: Niuchoutzu (1.1), Tingchuang (0), Mienchientso (2.7), Paimiaotzu (0).

ERHLIN CHEN: Hsichuangtzu—near Wanhsing (7), Liutzukou (0.3), Wanhsing (0.3), Hutso (0.3), Tutzulun (0), Chientsotzu (0.3), Chiuchaochia (0.7), Tientso (8), Shanliao (4), Houtso (0.4), Erhlin (0), Chuyuantzu—near Erhlin (0), Litoutso (0), Chuyuantzu—near Niuchoutzu (0.8), Hsichuangtzu—near Hsiaopuhsin (0).

FANGYUAN HSIANG: Tingwangkungliao (3), Hsiawangkungliao (4), Tsaohu (0.3).

TACHENG HSIANG: Tingshanchie (0).

The above list shows that among the 140 villages harboring *Oncomelania*,

snails in 75 ditches from 75 villages (more than half of the total villages) were, by the previously described procedure of examination, infected with cercariae. Out of the total number of 38,241 snails examined, 482 snails were found positive for schistosome cercariae. The average incidence of infected snails in this endemic area is, accordingly, 1.3 per cent.

The frequency distribution of the percentage of the infected snails in the 75 infected ditches is shown in Table 1. From this table, it is seen that the range of the percentage incidence of the infected snails in different infected ditches in this area is from 0.3 per cent to 30 per cent; the mean of the percentage incidence, 2.4 per cent; the median, 1.2 per cent; and the mode, 0.3 per cent. It shows further that above 78 per cent of the 75 infected ditches harbor 2 per cent or less of infected snails while relatively few ditches harbor high percentages.

TABLE 1

Percentages of Oncomelania *snails infected with schistosome cercariae in 75 infected ditches*

PERCENTAGE OF SNAILS INFECTED	NO. OF DITCHES WITH INFECTED SNAILS
Less than 1	35
1	11
2	13
3	6
4	3
5	1
6	1
7	2
8	1
9	1
30	1
Total	75

DISCUSSION

The results of our studies, as previously described, show that the habit of *O. formosana* in this endemic area, Changhua *Hsien*, differs in several important respects from that of other *Oncomelania* species, reported from the mainland of China, Japan and the Phillippines. The most obvious difference is that insofar as known from the literature, *Oncomelania* snails have never before been reported in ditches with turbid water of such high silt content. Moreover, their habitat is rarely so largely confined to the narrowest irrigation ditches of about 0.5 meter in width as in the case of *O. formosana* here. The present species is further characterized in this area by its peculiar association with the shade of the bamboo groves.

As to the distribution of *O. formosana* in Changhua *Hsien*, we have found that it is present in 140 villages which cover an area of 274 sq. kms. Among them, 138 villages are new records. Although the endemic area occupies the greater part of

the Changhua plain, it is interesting to note that the *Oncomelania*-positive villages are not evenly distributed, being more or less concentrated in the central part of the plain (Fig. 6). The factor or factors which operate to limit the spreading of this molluscan species are not definitely known and need further investigation.

Before the present investigation was carried out, infected *Oncomelania* snails were known in only one village. The present study reveals 74 more villages than in the old record. They are unevenly scattered throughout the area.

In studying schistosomiasis japonica, epidemiologists usually pay attention only to the incidence of human cases. As to the infection rate of the intermediate hosts, *Oncomelania* snails, in an endemic area, very little work has been done. But from the standpoint of technical convenience, it is actually easier to get the infection rate of the snail intermediate host than that of the human final host because the collection and examination of stools is a difficult procedure in the rural population of China. Under such circumstances, the infection rate of *Oncomelania* snails is a guide to that of human infection. However this investigation as a rule has only been casually done, giving the rate of infection of the snails for only a few ditches or canals. If the results are obtained from examining a large number of snail-harboring ditches or canals and the number of the *Oncomelania* snails examined from each ditch or canal are of statistical value, we can calculate the percentage of the ditches harboring infected snails, and get the average figure for the infected snails in the area concerned, and the range, the mean, the median and the mode of the incidence of infected snails in various infected ditches in an endemic district. With these data, the frequency distribution of the incidence of the infected snails in various snail-harboring ditches can be compared.

As to the incidence of cercarial infection of *Oncomelania* snails on the mainland of China, Mao (1948), reviewing from literature the hitherto known results of the dissection records of *Oncomelania* snails from 36 endemic areas in different parts of China, states that the range of incidence of infection is from 0.05 per cent to 40 per cent. The upper limit of the range in 11 endemic areas is given as 2 per cent. Abbott (1948) mentions that the infection rates of *O. hupensis* 10 miles west of Shanghai range from 0.0% to 10.5%. As to *O. nosophora*, Abbott says that the percentage of infected snails has never been as great as that found in *hupensis* or *quadrasi*. The senior author (Hsü, 1948), during his study of schistosomiasis japonica on the mainland of China in 1948, came across one very high incidence of infection of *O. hupensis* at Houpeng, Puyun *Chen*, Kahsing *Hsien*, Cheking Province. It is 49.6 per cent. This is a record for the highest incidence of cercarial infection of *Oncomelania* snails in the literature. However, such high incidence is extremely rare. The results of the present investigation in Formosa show that in the Changhua endemic area, on an average, the cercarial infection rate is 1.3 per cent for *O. formosana*, and in the infected ditches the range of incidence of infection is from 0.3 to 30 per cent with its mean at 2.4 per cent, median at 1.2 per cent and mode at 0.3 per cent. Judging from the incidence of infection of *O. formosana*, we may infer that the prevailing rate of the disease, schistosomiasis

japonica, in the endemic area in Formosa might not differ very much from that in the endemic areas on the mainland of China.

A study of the frequency distribution of the percentages of cercarial infection of *O. formosana* in 75 infected ditches reveals an interesting fact. The frequency curve of the percentage of infection, if plotted, will be a curve of positive skewness (see Table 1). In studying the frequency distribution of parasitic helminths in their naturally infected hosts, we (Li *et al.*, 1951) obtain a similar skew curve. Although these two studies do not belong to the same category, it shows, nevertheless, that this kind of frequency distribution is a common phenomenon in the parasitic world.

SUMMARY

1. Changhua *Hsien* is situated in the center of Formosa on the western seaboard and in the vicinity of the Tropic of Cancer. The arable lowland, being an alluvial deposit, is one of the most important rice growing centers in Formosa.

2. Regarding the snails, *O. formosana*, in this area, they are usually found in the small irrigation ditches of about 0.5 meter in width, especially in such ditches as are shaded by a row of bamboos. They can occasionally be found in ditches of about 1–2 meters in width, but very rarely in wider ditches. The fact that *O. formosana* lives in ditches irrigated by water of high turbidity deserves mention, as such a habit has never been recorded for the *Oncomelania* snails in other endemic areas in the Orient.

3. *O. formosana* was found in 140 out of 628 villages surveyed. They comprise an area of about 273 sq. kms., and are distributed mainly in the central part of this county.

4. Concerning the incidence of cercarial infection, 38,241 *O. formosana* from 140 ditches in 140 villages were examined (300 or less from each ditch). On the average, 1.3 per cent were positive, and in the infected ditches the range of infection was from 0.3 to 30 per cent, with its mean at 2.4 per cent, median at 1.2 per cent, and mode at 0.3 per cent. The frequency curve of the percentage of infection, if plotted, will be a curve of positive skewness.

5. The peculiar habits of *O. formosana* in this district, and the significance of the high incidence of infection of the snails in this county are discussed. Importance of the study of the incidence of infection of the *Oncomelania* snails in relation to the epidemiology of schistosomiasis japonica is emphasized.

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SOME OBSERVATIONS ON *DIPHYLLOBOTHRIUM LATUM* FROM SHAGWA LAKE, MINNESOTA

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Shagwa Lake (previously called Long Lake) at Ely, Minnesota has been known to show a high incidence of fish infected with plerocercoids of *Diphylobothrium latum* over a period of many years. The last reported survey made of this lake (July 1937) appears to be that published by Essex (1938). We had the opportunity of obtaining figures on the present status of the infection of fish in Shagwa Lake in August 1950 when we collected plerocercoids in order to obtain adult tapeworms to be used for physiological studies.

Approximately one week elapsed after the fish were caught before they were dissected; during this time they were packed in ice. The muscle tissue was teased apart with utmost care in order to discover as many larvae as possible. In many instances soaking the shredded muscle in saline facilitated the recovery of plerocercoids which might otherwise have been overlooked. Only larvae from the musculature are considered in this report; those recovered were found loosely embedded or attached. Table 1 shows the incidence and number of larvae encountered in the different species of fish in the present study, and for comparison those reported by Essex (1929, 1938).

It is apparent that the incidence of plerocercoids in the fish in Shagwa Lake is still being maintained at the high level reported in previous surveys. Magath (1929) found infections in every walleyed pike and great northern pike examined from Shagwa Lake, some with as many as 20 larvae, while Vergeer (1930) reported 2 of 4 perch from Shagwa Lake infected, one with 2 and the other with 3 plerocercoids.

The age of each fish dissected was determined by removing scales and examining them with the aid of a binocular dissecting microscope. It appears (Table 2) that the youngest fish, 2 to 3 years of age, harbored the fewest plerocercoids, and that the number of larvae tended to increase with the increase in age of the fish. The largest walleye examined was 18.3 inches. We have not been able to find comparable data in the literature, but Nicholson (1932) has reported that 52 percent of *Esox lucius* 10 to 18 inches in length, harbored *D. latum* plerocercoids, which were frequently very numerous. However, only 3 percent of the *Esox* over 24 inches long were parasitized.

Dogs obtained from Pennsylvania and examined repeatedly over a period of several weeks for *D. latum* eggs, with negative results, were given fresh larvae in gelatin capsules intra-esophageally; the infection results are shown in Table 3 (dogs 1 through 8). Fecal examinations were made daily for eggs beginning on the ninth day and were continued until found positive or until necropsy. As the table

[1] The authors wish to thank Drs. Eloise B. Cram and M. S. Ferguson for aid in the dissection of the fish.

indicates, 6 of 8 dogs developed the infection as evidenced by the passage of eggs. However, at necropsy of all the animals on the twenty-eighth day, adult worms were recovered from only 4 of the 6 positive dogs. The worms of the two "negative" dogs which had previously passed eggs were probably expelled within a day or two previous to necropsy, since one of the animals still showed great numbers of eggs in the fecal material obtained from the colon when the animal was sacrificed. It was found that 2- to 3-year old dogs took the infections as readily as younger ones only 5 months of age. One of the dogs infected with *D. latum* har-

TABLE 1

Incidence of D. latum plerocercoids in fish from Long Lake, Minnesota, August, 1950

SPECIES	NO. FISH EXAM.	AVER. SIZE FISH (RANGE)	NO. POS.	PER CENT POS.	TOTAL NO. PLERO-CERC.	AVER. NO. PLERO-CERC. PER FISH	RANGE IN NO. OF PLERO-CERC.
1. *Stizostedion vitreum* (walleyed pike)	65 (6; 15)*	14.3″ (6.8″–18.3″)	53 (6; 15)	81.5	180	2.8	1–21
2. *Esox lucius* (great northern pike)	11 (12; 4)	20.6″ (14.1″–25.7″)	11 (12; 4)	100	72	6.5	1–17
3. *Perca flavescens* (yellow perch)	10 (10; 2)	10.3″ (9.4″–12.0″)	1 (6; 0)	10	1	0.1	—
4. *Leucichthys artedi tul libee* (tullibee)	6	—	0	—	—	—	—

* Figures in parentheses refer to findings of Essex, first figure in parentheses obtained previous to 1930; second from 1937 survey.

TABLE 2

Average number plerocercoids per age of walleye in years

AGE IN YEARS	NO. FISH	MEAN NO. OF LARVAE
2	3	1.0
3	18	1.7
4	18	3.0
5	11	2.5
6	8	4.6
7	3	3.7
8	1	21.0

bored concomitantly several *Dipylidium caninum*, while one of the negative animals contained a single specimen of *Taenia pisiformis*. Of the total of 37 larvae fed to the 4 dogs from which worms were ultimately recovered, 15 (40.5 per cent) developed to adults.

Magath (1929) fed 9 dogs larvae from fish from Burntside and Shagwa Lakes, and infected all. Of a total of 22 larvae fed, 17 worms were recovered. Vergeer (1929) fed 7 dogs with a total of 24 plerocercoids obtained from Canadian fish; 6 eventually were found positive by the recovery of worms or of eggs. Worms

were found in 4 of these dogs, each of which harbored a single specimen. These 4 worms developed from a total of 20 larvae fed, the dogs individually having received 2, 8, 4, and 6 plerocercoids.

In the present study, 3 guinea pigs, 3 rabbits, and 4 rats were given 4 plerocercoids each, except for 1 of the rats which received only 2 larvae. In addition, an adult rhesus monkey was given 6 larvae. The rats, rabbits, and guinea pigs were sacrificed between the fourth and eleventh days following feeding, and the intestinal tracts carefully examined along their entire lengths, but no worms were found. Stool examinations were performed daily on the monkey beginning with the ninth day after feeding until it was sacrificed on the twenty-seventh day. Again, no eggs or worms were recovered. Ward (1929) reported that he successfully infected dogs with plerocercoids from the Ely area but could not obtain infections of young kittens, full grown cats, or guinea pigs. Wardle (1935) on the other hand, reported definite development in the cat, but noted that the worms do not grow rapidly in this host, and maturity may not be established even after

TABLE 3

Infection of dogs with plerocercoids of D. latum

DOG NO.	NO. PLEROCERC. FED	NO. DAYS TO EGG PASSAGE	NO. WORMS AT NECROPSY
1	7	20	4
2	11	No eggs found	0
3	7	14	0
4	11	20	0
5	10	16	3
6	10	16	4
7	10	14	4
8	6	No eggs found	0
9*	15	18	4

* Plerocercoids from fish stored at low temperature at least 42 days.

6 weeks. He expressed doubt whether functional or morphological maturity is reached in hosts other than man, bear, or dog.

As indicated in table 3, the initiation of egg passage in the stool of infected animals ranged from the fourteenth to twentieth day after feeding of larvae, averaging 16.6 days. The reports concerning the first appearance of eggs in the feces of dogs are variable. Ward (1929) stated that eggs of the parasite were evacuated in the feces 4 weeks after the plerocercoids were fed. Magath (1929) reported that eggs can be found in the stool 25 days after dogs are fed larvae. An analysis of his data indicates that in his group of 10 infected dogs, the beginning of egg passage ranged from the fourteenth to thirty-eighth day after larvae were fed, averaging 26.5 days. Wardle (1935) indicated that in the dog, the period after initial infection when eggs appear in the feces was 19 to 25 days. Nicholson (1928) reported that more than 5 weeks elapsed after infection before the appearance of eggs in the stool of a dog in which many (19) worms developed.

Although there is information present in the literature concerning the effects of

low temperature on *D. latum* plerocercoids for relatively short periods of time, there does not appear to be much information concerning the effects of rather prolonged storage at low temperature. Kjava (1913) in Finland, found that larvae within the fish were killed by a 48-hour exposure of the fish to an external temperature of −9°C., and remained viable within the fish at environmental temperatures between 3° to 50°C. Magath and Essex (1931) found that when 10 larvae that had been kept in fish at −10°C. for 88, 40, and 16 hours respectively were fed to 3 dogs, no worms developed. Nicholson's (1932) studies revealed that larvae remained alive in fish stored for 24 hours with the minimum temperature falling to only −3°C., whereas all were killed in fish stored for 24 hours with the minimum temperature reaching −11°C. Wardle (1933) examined the effects of different temperatures over relatively short periods and characterized the extremes in the temperature range within which survival was possible. For the lowest temperature tested, −10°C., death of larvae in muscle occurred in less than 1 hour, as judged by the response of the worms to tactile stimuli when brought to a temperature of 38°C.

In the present study only limited observations on temperature effects could be made. A walleyed pike which was stored under ice in a refrigerator room which ranged in temperature from −2°C. to 5°C. for at least 42, and possibly 46, days after removal from the lake, showed a heavy, slimy, fungoid growth on the outer surface. Fifteen plerocercoids were recovered from the muscles. Most of the larvae exhibited some degree of movement in warm saline, but were rather feeble when compared to the vigorous activity of those dissected from fish that had been iced for only a week. All, however, appeared normal morphologically. The 15 larvae were given to a dog; eggs were found in the feces 18 days later. When a necropsy was performed on the animal on the twenty-second day, 4 adult worms were recovered. The percentage of larvae developing is therefore almost as good as that obtained with larvae from fish that were iced for only a few days. These results are of interest in view of Wardle's (1935) findings that the most favorable temperature values for *D. latum* plerocercoids lie between 38°C. and −2.8°C. He states that, though proven, it is possible that the low body temperatures of the fish in winter are unfavorable to the plerocercoids and that a proportion of them die naturally within the fish muscle. It would appear from the present results, however, that the larvae can certainly maintain themselves for an appreciable period of time at temperatures even lower than what would normally be encountered by infected fish in nature. Further physiological data on this very interesting phase in plerocercoid biology are highly desirable in order to understand the factors that may be involved in the death of larvae which is observed to occur in fish during the winter months.

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* Not consulted in the original.

DIETHYLCARBAMAZINE (HETRAZAN) IN EXPERIMENTAL TRICHINOSIS[1]

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The general acceptance of diethylcarbamazine or Hetrazan (1-diethylcarbamyl-4-methylpiperazine hydrochloride [or dihydrogen citrate]) as a useful drug in the treatment of filarial diseases caused by several types of worm and affecting various animals, including man, has stimulated its trial in treatment of other nematode infections. It has been claimed by Oliver-González, Santiago-Stevenson and Hewitt (1949) and by Etteldorf and Crawford (1950) to be effective in the treatment of infections with *Ascaris lumbricoides* and by Hewitt, Wallace, White and SubbaRow (1948) to be effective in the removal of ascarids (species not mentioned) from dogs. It was without effect on the tapeworms, hookworms and whipworms of dogs. In reports by van de Erve (1949) ,and also by Horton (1950), diethylcarbamazine was said to be curative of larva migrans caused by *Ancylostoma braziliense* in man, but the proof of specific larvicidal action is not evident from these articles.

The mode of action on nematodes is poorly understood even now, after about five years of intensive study by many investigators. In the case of Ascaris there can be no doubt but that any action is on the adult worm and, in the case of *Ancylostoma braziliense*, on the larval form. One may conclude from the evidence available that the effect on filarial worms is exerted at both the adult and the larval stages—yet few authors have definitely committed themselves on this point. According to Hewitt, and others (1948), two hours after 25 mg/kg. (25 mg. per kilogram of body weight) of diethylcarbamazine has been given to cotton rats, the number of microfilariae in the peripheral blood is reduced 90 per cent. In a number of rats, even after the reduction in microfilariae had taken place, no evidence of dead adult worms could be found. This evidence could be interpreted as indicating destruction of the larvae. On the other hand, the weight of discussion in most published papers indicates that the major effect is destruction of adult worms. Indeed, if this did not take place, the conclusion drawn from reexamination of persons in mass treatment experiments would be unwarranted. The conclusion is that the microfilariae in human beings have been permanently reduced in number or eliminated. Unfortunately, a control series cannot be found in the evidence presented to date. It would be useful to have a report of a series of untreated patients who had been as carefully studied as the series of treated patients. Certainly a series in which the larval counts had been done daily or weekly over a long period of time before treatment had been started, instead of only a single count in each case before treatment, would solve several problems.

[1] Read before The American Society of Tropical Medicine, Chicago, Illinois, November 15, 1951.

[2] Sections of Clinical Pathology and Biochemistry.

[3] Fellow in Parasitology.

Statistical treatment of the data also would be useful, especially a study to reveal the accuracy of the larval count in the aliquot of blood used for the test.

While it is not the purpose in this paper to question the validity of claims as to the curative effect of diethylcarbamazine in filarial diseases, it is interesting, in view of our results, to call attention to the numerous drugs which have been advocated for the treatment of filariasis and the supporting evidence advanced for each such claim. Yet filariasis is still far from a conquered disease and little headway was claimed before the use of diethylcarbamazine. As recently as 1944, Brown (1944) reported microfilarial reductions of 85 to 100 per cent in a series of cases in which lithium antimony thiomalate was used, and Brown and Sheldon (1941) reported that 5 dogs out of 6 were cured of heartworm by the use of sulfanilamide and Fuadin. In the Conference on the Chemotherapy of Filariasis held in 1947, many drugs were reported to be highly effective against various species of filarial worms; however, the ease of administering diethylcarbamazine, its small dosage, the lack of serious or even moderate reactions and the fact that results were as good as, or better than, those obtained with other drugs has definitely placed it in a favored position.

In the treatment of trichinosis, an equally long list of drugs could be assembled and many claims for their curative effects could be made. Yet today there is no drug which will stand the test of critical examination and no one can offer a victim of the disease any but palliative treatment; the disease runs its course. Some of these drugs have been discussed by Magath (1926) and many others have been added to the list since.

McCoy (1938) investigated the effect of sulfanilamide in the treatment of trichinosis of rats and found it to be of no value. McNaught, Beard and DeEds (1939) and Riedel and Lunde (1949)[4], on the other hand, found that this drug reduced, by about half, the number of larvae in the musles of rats and mice.

Oliver-González and Hewitt (1947) tested the effect of diethylcarbamazine on the intestinal phase of trichinosis in rats. The procedure was to feed rats either 1,000 or 1,300 larvae and to begin administering 200 mg/kg. three times daily by stomach tube after twenty-four hours had elapsed. Six rats were treated five days; then they and a like number of controls were examined to determine the recovery of worms from the intestine. Six more were treated ten days and examined, as were controls. In these two sets of animals, the controls yielded about four or five times[5] as many worms as the treated animals. The figures given are

[4] In this report there is no statistical treatment of the data although the results are expressed in percentages. A total of 12 animals were used as controls and the variability in the number of larvae recovered is very great. Some of the control animals revealed no more worms than animals of the treated group of which only 11 were used in the series which received sulfanilamide. The variation, which is inevitable in an experiment of this nature, makes it necessary to have large series of animals and to treat the results by critical application of statistical methods. In Riedel and Lunde's table 2 the value of p is 0.04 for the sulfanilamide group and would be considered significant, but the p value for this drug in table 4 is M 0.01 and is not significant. Even this statistical test is not sufficient where the ranges of distribution are so great.

[5] There is either a typographical error in the published table or the conclusions in the text are incorrect.

the averages and no ranges are given. In a second series three sets of 6 animals each were treated for five, ten and twenty days; after thirty days they were killed and the larvae in the muscles were counted. The first 6 animals averaged 320 larvae and the controls, 31,200; the second group averaged 112 and the controls, 26,100; the third group revealed an average of 2 larvae; there were no controls for this group. The authors concluded that the number of adult worms in the treated animals was reduced and hence the number of larvae in the muscles was reduced. They suggested that the mg/kg. dose for man might be less than that for rats, judging from work previously reported on filarial worms.

It is difficult to evaluate conclusively these results without the complete protocol. In feeding rats with larvae of trichina there is great variability in the number of larvae that can be recovered from the muscles and averages are of doubtful value. Mathieson (8), in a long series of rats, to each of which he fed 2,000 worms, found variations in recovered larvae ranging from 5,000 to 75,000. Some rats, in spite of meticulous care in feeding and recovery, revealed no infection at all. Other investigators have found wide variations in the number of larvae in the muscles of experimental animals when attempts were made to hold the dosage constant. Riedel (1948) showed that age played a role, McNaught and others (1939) thought the female more resistant than the male and Miller and co-authors (1932) expressed the belief that biologic variations in the host played an important role. It would appear, however, that in so far as the experiments of Oliver-González and Hewitt went, some beneficial effect of the drug was demonstrated. It is not evident, however, that this effect was solely manifested on the intestinal phase of the worm. The dosages given were enormous as compared to those given to man or those used to control *Litomosoides carinii* in cotton rats or *Dirofilaria immitis* in dogs.

EXPERIMENTAL PROCEDURE

A white rat[6] infected with *Trichinella spiralis* was killed and the diaphragm was fed, on February 22, 1951, to a number of rats in order to establish a stock of infected animals.

(A1) Five of these rats were given 25 mg/kg. of Hetrazan[7] twice daily for about two weeks beginning May 6 (Table 1). Five similar rats were used as controls and were killed on the same day.

On May 4, 4 rats, yielding 210 gm. of ground muscle, were used for preparation of larvae by the customary digestion method. The larvae were fed by stomach tube to 15 rats on May 5 so that each received approximately 25 larvae. The fifteen rats of this series were retained as controls for experiments B1 and B2. Three of these 15 rats were killed on May 20 and the rest on May 27.

(B1) Twenty-one rats were given 25 mg/kg. of diethylcarbamazine twice a

[6] This rat was kindly supplied by Dr. W. H. Wright of the National Institute of Health, Bethesda, Maryland.

[7] Kindly furnished by Dr. S. M. Hardy of Lederle Laboratories Division, American Cyanamid Company. The salt used was 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate.

day beginning May 6 for about three weeks. At the end of that time all the rats were examined.

(B2) Twenty rats were given 25 mg/kg. of diethylcarbamazine twice daily beginning May 12 for about three weeks. The rats were examined on June 3.

(C1) On June 26, thirty-five rats were fed small portions of a heavily infected diaphragm from a stock rat. The dose given to each rat was approximately 150 larvae. On July 9 each of 25 rats was given 50 mg/kg. of diethylcarbamazine twice daily for about two weeks. Ten rats were retained as controls. All were killed and examined on July 23.

TABLE 1

Summary of experiments

EXPERIMENT	NUMBER OF RATS	LARVAE FED	TREATMENT BEGUN	TREATMENT ENDED	DAILY DOSE mg/kg.	EXAMINED	RESULTS INFECTION
A1	5	2/22			none	5/20	Heavy
	5	2/22	5/6	5/20	50	5/20	Heavy
B1	21	5/5	5/6	5/27	50	5/27	9 heavy, 4 medium, 3 light, 5 negative
B2	20	5/5	5/12	6/3	50	6/3	7 heavy, 4 medium, 4 light, 5 negative
Controls for B1 to B2	3	5/5			none	5/20	Negative
	12	5/5			none	5/27	3 heavy, 2 medium, 5 light, 2 negative
C1	25	6/26	7/9	7/23	100	7/23	24 heavy, 1 medium
Controls for C1	10	6/26			none	7/23	9 heavy, 1 medium

From each group of rats of each experiment, pieces of infected diaphragms which were positive were fed back to clean rats in order to see if the larvae were viable. Each of the rats used weighed about 125 gm. when fed and, during a month's observation, gained about 100 gm.

The method of examination was to kill the rats with ether and then to press out the entire diaphragm in a muscle press and examine it under a wide-field binocular at 50× magnification. If no larvae were seen, intercostal muscles and muscles of the extremities were examined. When the larvae were so thick that the cysts were separated by no more than the length of the cyst, the infection was graded as "heavy". A "light" infection was used to designate infection of less than 10 larvae per diaphragm and "medium" infections were those graded between "heavy" and "light".

Diethylcarbamazine was prepared in sterile distilled water and injected intraperitoneally twice daily.

DISCUSSION

The experiments were partly designed to reveal the effect of diethylcarbamazine on experimental trichinosis of rats when the drug was given with relation to various stages in the development of the disease. In this manner it was hoped that it could be learned whether the effect was exercised on the adult worms, the larvae while still wandering in the muscles or the larvae after early encystment.

In experiment A1, in which encystment (but with little or no calcification present) had taken place, there was obviously no effect on the larvae and although the larvae appeared normal, they were fed back to rats and produced heavy infections.

In experiment B1 the number of larvae fed was obviously minimal and the treatment was begun immediately so that any effect would be due to action on the adult or very early larval stage. In experiment B2 the delay in treatment was calculated to be effective on the young larvae while still wandering or on worms very early after encystment. Here again the drug was without detectable effect.

In experiment C1 the conditions of B2 were in general repeated but the doses of larvae were larger and were fed by a more "natural" method; also the dose of diethylcarbamazine was doubled. As in the other experiments the drug produced no demonstrable effect. Back feeding of larvae from rats of experiments B1, B2 and C1 demonstrated that the larvae not only were viable but were capable of producing trichinosis.

The main purpose in the design of the experiments was to give a practical clue as to the usefulness of the drug in human trichinosis. In this disease, for obvious reasons, one cannot administer the drug beginning with the day the patient eats the infected meat, nor does one ingest larvae freed of their cyst walls. Thus the dosage and the conditions, especially in experiment C1, do approximate the conditions under which man might be treated. The dosages we used were fifteen to thirty times the daily dose used in the treatment of filariasis of man and were administered almost twice as long. Even these doses did not result in any effect on the disease although the drug was administered intraperitoneally.

These experiments appear to indicate that diethylcarbamazine will not be useful for trichinosis of human beings. If the literature is to be taken at face value, and if the drug has any beneficial effect in infections caused by animal parasites, it must be directly on the parasite. In the literature concerned with the filarial worms it frequently is pointed out that the dose for man is smaller than that for animals. However, here the species of parasites which infect man and animals are different while in trichinosis the species is the same. Thus, if the dose used in these experiments will not affect *Trichinella spiralis* in rats

there is no reason to believe a smaller dose will affect the parasite in man. Moreover, doses of the magnitude used in our experiments have not been given to man to our knowledge and would be unwarranted without further experimentation. While our experiments were designed to show the effect on various stages of the disease, from a practical standpoint no drug will have any value for human beings unless it will destroy the larvae during their migration and early encystment. Even in these stages of the disease the diagnosis rarely is made. Usually the patient has been ill for at least three weeks before the disease is even suspected, and diagnoses made prior to ten days after onset of the illness are indeed rare. After the parasites have become encysted, the illness subsides rapidly and when much calcium has been deposited in the cysts the acute symptoms have subsided and the danger of a fatal outcome is minimal.

CONCLUSION

Large and excessive doses of diethylcarbamazine (Hetrazan), given under the conditions of the experiments described, did not affect the course of experimental trichinosis in the rat nor was there evidence that, in this animal, the drug had any demonstrable effect *in vivo* on the adults or on the larvae, migrating or encysted, of *Trichinella spiralis*.

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OBSERVATIONS ON MOSQUITO DENSITY IN AN ENDEMIC MALARIOUS AREA IN EASTERN COLOMBIA

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We have recently shown the feasibility of a close study of mosquito behavior and mosquito density in the savanna country of Eastern Colombia. The observations published in our first report (de Zulueta, 1950) were made in an area thinly inhabited, with a few scattered cattle ranches and a low cattle density; care was taken, however, to make the observations far away from human dwellings and free from herds of cattle. The results reflected the conditions in which mosquitoes live free from man and domestic animals.

We were interested to know how the presence of man with his agricultural activities and domestic animals would influence the mosquito density and the mosquito bionomics in general in the savanna country. For that purpose we selected a small settlement, well-known as an endemic malarious area, haunted by *Anopheles darlingi*, the chief malaria vector of this region as shown by Renjifo (1948).

THE STUDY AREA

The San Antonio settlement has developed in the last five years in an area previously devoted to cattle breeding in which an increasing number of immigrants from other parts of the country are now working the land. It is situated 70 km. south of Villavicencio, and only 40 km. from the foot of the eastern range of the Andes. The settlement has developed in an area of savannas intersected by forest galleries. An important feature is the vicinity of a large area of forest 20 to 30 km. wide, stretching along the Ariari river and its affluents. In this forest, the settlers have made clearings and are engaged in raising crops such as rice and maize. This area has the same climatic features as the Villavicencio region, which have been previously described by Bates (1948) and Bates and de Zulueta (1949). Characteristic of all this region is the alternation of a rainy season lasting from April through November, and a dry season covering the remaining months. Rainfall in Villavicencio, situated at the very foot of the Andes, reaches 4,500 mm. per year. Precipitation away from the mountains is less abundant, but it is always well over 2,500 mm. per year.

The general features of the countryside in San Antonio are very much the same as those described in our previous paper on the habits of savanna mosquitoes (de Zulueta, 1950) to which the reader is referred for more information. The mosquito fauna of this area includes the representatives of the typical savanna mosquitoes, such as *A. pessoai*, *A. peryassui* and *A. parvus*, but being not far from the mountain front it has several species characteristic of the piedmont area, the transition area between the mountain slopes and the savanna country of the Orinoco system. Characteristic of this transition area is *A. darlingi*, the

chief malaria vector of this region, which is seldom found in the vast plains of the Eastern Llanos, with the exception of some forest galleries along the rivers like the Meta and Orinoco. Another anopheline found abundantly in the San Antonio area is *A. argyritarsis*, also seldom found in the true Llanos.

The San Antonio settlement has about 600 inhabitants living in "ranchos" or huts and small houses scattered mainly over a stretch of savanna of some 5 km. length by 1 or 2 km. wide, where all the observations reported in this paper were carried out. All these dwellings are built in the savanna, and most of them at the edge of the great forest area of the Ariari river. The houses are seldom

FIG. 1. A typical human dwelling in the San Antonio study area, used as capture station. Beyond the savanna can be seen the edge of the Ariari forest.

close together, and are usually separated by several hundred metres of savanna Most of the peasant dwellings are of the "llanero" type, built on poles covered with a thatched roof made usually of Mauritius palm leaves and with walls of wooden planks, bamboos or dried mud and straw. It must be noticed that in none of these buildings do the walls reach the roof and there is never a proper ceiling. Such primitive rooms are thus open to the air, and in many cases the little "ranchos" are no more than a shelter, that is, a thatched roof and the necessary supporting poles and beams from which the peasants sling their hammocks. Furthermore, the walls, if they exist, are seldom well finished, having cracks and openings between the planks and materials used in building, and are not painted or white-washed, as is usually the case with the typical "llanero ranchos". This is

due to the provisional character of the dwellings, the San Antonio settlement being inhabited by immigrants recently arrived from other parts of the country. A typical example of these houses is given in Figure 1, one of our stations for mosquito house captures.

Malaria surveys have been carried out in the San Antonio area since 1948. There being no school where the children could be examined, a sample taken at random among the general population was examined. The last two surveys made in the area gave the following results:

DATE	PEOPLE EXAMINED	SPLEEN INDEX	PARASITE INDEX
September 1949	170	46.4	24.1
June 1950	155	43.9	31.0

The two surveys were carried out during the rainy season, prior to the DDT house-spraying undertaken in this area. The fact that the majority of the population of San Antonio are newcomers, many of them from the highlands of this country free from malaria, and therefore with no acquired immunity, has a bearing no doubt on the high malaria indexes. In neighboring areas with a more stable population, the malaria indexes are lower, though the mosquito fauna and the general features of the country are very much the same. The type of dwelling may also have some importance and the lack of walls and ceilings is undoubtedly a handicap in malaria control by house spraying; but the San Antonio settlement is a typical example of the conditions met with in many places of Eastern Colombia, where the fertility of the soil attracts immigrant peasants and where malaria is the main obstacle to the development of new communities.

METHODS

The capture of mosquitoes in houses was an essential part of our program of work, but, from what has been said above, it can be deduced that it presented certain difficulties. The fact that there were not properly closed rooms made it difficult to use such methods as window traps and killing sprays, widely used elsewhere. Furthermore, as will be shown later, the house-haunting mosquitoes of this region do not stay in the houses after sunrise. Mosquitoes had to be caught, therefore, during the night at the time of biting, in order to obtain a proper sample. As we found that the greatest mosquito activity took place between 6 P.M. and 9 P.M., mosquito captures in the houses were reduced to those three hours.

Three "ranchos" of the average type were selected for these experiments. The human bait was made up of the inhabitants plus two laboratory workers for each house in charge of the mosquito captures. The total number of persons was six in two huts and five in the third one. The workers were supplied with dark blue overalls, flash-lights and leather bags containing a sufficient number of cotton plugged test-tubes for each trial.

The normal procedure was for the two laboratory assistants to sit in the middle

of the peasant family, leaving them eating or engaged in their usual occupations, while they themselves, with trousers rolled up to the knees, caught the mosquitoes in the test-tubes with the aid of flash-lights as they came to bite. Experience showed that they bit mainly on the legs. A tube was used for each mosquito and the material collected was identified next morning.

Stable trap captures were made to obtain comparative results, using two identical traps of the type described by Bates (1944) as the "Egyptian trap". This type of trap has been used by the Villavicencio laboratory for many years with satisfactory results. The stable traps were usually baited with donkeys, but in some experiments we used calf and man as bait. In this last case a canvas bed was

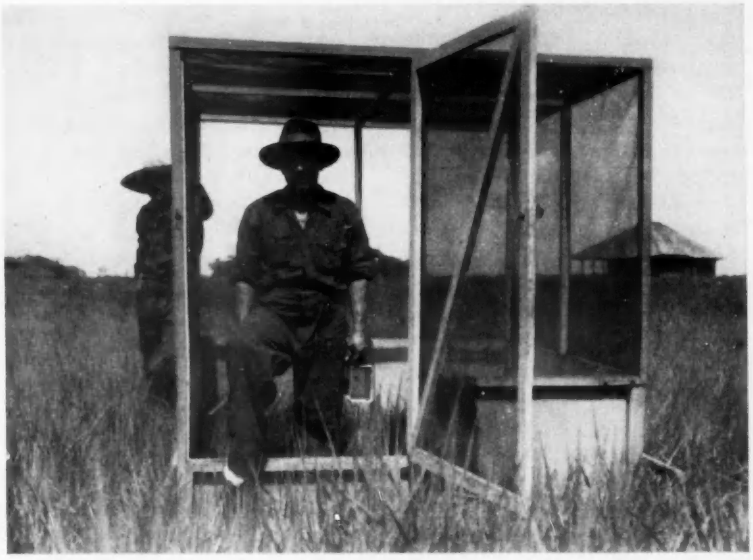

FIG. 2. The portable cage for catching resting mosquitoes in the daytime, set up in the savanna with its door open. The hut in the background is one of our capture stations.

put inside a trap and a mosquito net was extended over the bed, leaving enough room for the mosquitoes in the upper part of the trap.

The traps were usually placed in an ordinary stretch of savanna, but in some experiments the grass was cut around one of the traps, thus making an articial clearing. Hoeing the grass by manual labor to leave the ground bare proved to be a laborious procedure. Not having at our disposal an adequate implement, we used instead an improvised machine consisting of a steel frame supporting a wooden box with a piece of rail sharpened at the edge in front. The wooden box being loaded with a sufficient weight of sand, the sharp edge of the rail would take up the grass by the roots when the machine was pulled by a truck.

In our previous paper on the habits of the savanna mosquitoes (de Zulueta,

1950), a method was described for catching the mosquitoes resting in the grass during the daytime. A 4 sq. m. muslin tent, suspended by four poles, was used to enclose an area of savanna and collect the mosquitoes resting there, after spraying the grass with a repellent such as citronella. Each capture took from 15 to 20 minutes. In order to shorten this procedure, a portable cage was built of aluminum and screening with a light frame of wood and a total weight of less than 40 kg. The cage was fitted with a door and handles to facilitate the work, so that captures could be made regularly every 10 minutes. The cage is reproduced in Figure 2 and, being 2 m. high, it allows people to stand upright and at the same time to catch the mosquitoes easily on the ceiling. Being 1.73 m. on a side, it covers almost exactly 3 sq. m. of ground.

As a complement to these methods of capture, we used nets fixed to a moving vehicle for catching flying mosquitoes in the evening. The technique is described in detail and illustrated with figures in one of our previous papers (de Zulueta, 1950). All the captures reported in this paper were made between 6:10 and 6:30 P.M., the car traveling at an average speed of 30 km. per hour, thus covering a distance of 10 km. Two strong muslin nets with a circular metal frame 60 cm. in diameter were fixed to poles attached to the car; the nets were set at a height of 1.5 and 4.5 m. above the ground.

The work reported in this paper was done during November 1949 and May to August 1950, all wet months with high mosquito density. The reader must be reminded here that all the work reported in this paper was done with limited equipment and with a few assistants, never more than 6 at a time.

House captures

These mosquito catches were made between 6 and 9 P.M. in the huts chosen as good examples of the average type of local dwelling. In one of the huts (No. 1), having a single room with incomplete walls, the inhabitants and the men making the captures sat inside on benches. In the other two huts the men sat with the occupants at the entrance. In No. 1 hut, mosquitoes had to penetrate further into the house than in Nos. 2 and 3, but to apply the terms "indoor" and "outdoor" to the catches would be misleading, since these "ranchos" had no doors and only incomplete walls. A summary of these captures appears in Table I. It shows that the results obtained in the three capture stations were very much the same.

The men making these captures were exchanged every night from one hut to another; it was soon noticed that the attractiveness of the men varied considerably and was independent of the hut in which they were and of their position in it. The difference in the attractiveness of individuals to mosquitoes has often been observed and is clearly shown in the results of Ribbands (1949). To avoid differences other than individual attractiveness, all the men making the captures had a bath at the same time in the afternoon, using the same brand of soap and putting on clean overalls. The results obtained under such uniform conditions, in four captures by four men, are given in Table II, and they confirm the wide difference in attractiveness shown by different individuals. It will be noticed that

the size and weight of the four men engaged was about the same. The results showed also that their degree of attractiveness was the same for the two main house haunting anophelines of the region: *A. darlingi* and *A. pessoai*.

Table IX shows that the culicines coming into the houses to bite were only 2.1 per cent of the total catch. The anophelines thus formed the bulk of these house captures, their distribution by species being given in per cent figures in Table X. *A. darlingi* was the most frequently caught mosquito in houses, with 70.0 per cent of the total anopheline catch. *A. pessoai* came second with 27.5 per cent; the other anopheline species together amounted to only 2.5 per cent of the total anopheline capture.

The fact that the culicines were so few in these house captures is worth noticing. It must be remembered that *C. fatigans*, the commonest mosquito nuisance

TABLE I

Results of 32 house captures made between 6 and 9 P.M. in 3 capture stations; (based on 1,185 mosquito specimens)

CAPTURE STATIONS	NUMBER OF TRIALS	HUMAN BAIT (NUMBER OF PERSONS)	MEN CAPTURING	MOSQUITOES CAPTURED		MOSQUITOES PER MAN-HOUR
				Anophelines	Culicines	
Hut No. 1	8	6	2	295	1	6.16
Hut No. 2	16	5	2	477	17	5.15
Hut No. 3	8	6	2	389	6	8.23

TABLE II

Mosquitoes caught biting 4 men in 4 house captures

MEN	*A. pessoai*	*A. darlingi*	MISCELLANEOUS	TOTAL ANOPHELINES	CULICINES
R. P.	8	18	0	26	1
P. B.	15	46	3	64	0
C. B.	24	52	2	78	1
M. B.	29	108	4	141	0

in the villages of Eastern Colombia, is not found in the scattered dwellings in rural areas, its character thus being more urban than really domestic.

We were interested in knowing how the species composition changed during the three hours of these evening captures. The results of these serial captures are summarized in Table III. They show that *darlingi* increases in numbers from 6 to 9 P.M. and that *pessoai* seems to have its greatest activity between 7 and 8 P.M. *Argyritarsis* and the other anopheline species seem to approach the houses only during the first two evening hours. This confirms our experience in other places in Eastern Colombia, where *darlingi* is found active in houses all through the night, whereas the other house haunting anophelines drop after the peak of the evening hours. Table V, summarizing results of stable trap captures with animal bait, shows that the majority of mosquitoes were captured before 9 P.M. but that a greater number of *darlingi* can be captured between 9 P.M.

and 6 A.M. than during the first three hours of the night. It is thus likely that if our house captures had been carried on all through the night, the percentage of *darlingi* would have been even higher than is shown in our results.

During these captures it was noticed that mosquito activity dropped as the wind increased and was stopped altogether by rain. It was also observed that the mosquitoes came in "waves", and that after considerable catching activity there was a lull of several minutes before a new "wave" of mosquitoes entered the house.

TABLE III

Anopheline mosquitoes captured in human dwellings at different hours; (based on 948 specimens)

TIME	*A. pessoai*	*A. darlingi*	*A. argyritarsis*	MISCELLANEOUS
6 to 7 P.M.	81	168	7	11
7 to 8 P.M.	103	201	1	4
8 to 9 P.M.	73	299	0	0

TABLE IV

Comparative results of 28 trials made with stable traps in an artificial clearing and in a control area; (based on 10,220 specimens)

AREA OF CAPTURE	CULICINES CAPTURED	ANOPHELINES CAPTURED	DISTRIBUTION BY SPECIES			
			A. pessoai	*A. darlingi*	*A. argyritarsis*	Miscellaneous
Cleared area	1,191	1,191	1,052	116	13	9
Control area	4,388	3,451	3,154	95	182	20

TABLE V

Results of comparative evening and night stable trap captures; (based on 669 specimens)

TIME OF CAPTURE	NO. OF TRIALS	MOSQUITOES PER TRIAL	MOSQUITOES CAPTURED					
			Culicines	*A. pessoai*	*A. darlingi*	*A. argyritarsis*	Miscellaneous	Total anophel.
6 to 9 P.M.	4	85.0	113	222	2	2	1	227
9 P.M. to 6 A.M.	4	82.3	187	130	11	0	1	142

These captures were supplemented by careful search in the morning for mosquitoes resting in the houses, but we could only occasionally find a few engorged females, usually *darlingi* females, which had got into mosquito nets through some opening or under the skirts, improperly folded, and were therefore unable to leave the house at sunrise. We never found a single mosquito resting on the walls or in dark corners of these peasant huts in the day-time. This confirms our experience in other parts of Eastern Colombia where no anophelines are found resting in the houses during the day.

In the course of our evening house captures, we observed that, after biting,

the *darlingi* and *pessoai* females would sometimes, if allowed to fly, go to rest on the wood or mud walls for a few minutes. The time of rest was always short before they again flew into the open and it was found also that in many cases the mosquitoes would come directly to bite and leave the house without resting on the walls. During these observations, no mosquito males were seen coming into the houses with the blood seeking females.

Stable trap captures

The two traps used in the present observations were set up in the open savanna at a distance of about 500 m. from the "ranchos" used as capture stations, the distance between the two stable traps being approximately 100 m. It became apparent from our first results that the species composition in our stable trap captures was widely different from that of our house captures. This could be attributed mainly to the difference of bait or to the difference between the houses and the traps. To test the effect of the bait, a series of comparative catches was made using man, calf and donkey alternately as bait. In each trial, the baits

TABLE VI

Results of comparative stable trap captures using man, donkey and calf as bait; (based on 4,501 specimens)

BAIT	NO. OF TRIALS	MOSQUITOES PER TRIAL	MOSQUITOES CAPTURED	
			Culicines	Anophelines
Man	6	48.0	52	236
Donkey	2	579.0	2,413	642
Calf	4	763.8	360	798

were exchanged to avoid the effect of the location of the traps. The bait was put into the traps before 6 P.M. and the mosquitoes removed next morning after 6 A.M. The general results of these trials appear in Table VI. As can be seen, a man was less attractive to mosquitoes than a donkey and much less than the calf used in these experiments, which was heavier than the donkey. In a previous paper (de Zulueta, 1950) we observed that the number of mosquitoes caught was in proportion to the size and bulk of the bait. This seems to be confirmed by the results we are discussing here. But in those experiments we found that the species composition was the same using donkeys, calves and chickens as bait. In the present case, however, there can be no doubt that the type of bait affects the species composition. It is clear that the culicine mosquitoes are much more attracted by calf than by donkey or man, as can be seen in Tables VI and IX and, as regard the anophelines, our results show that the attractiveness of man to *A. darlingi* is several times higher than that of either donkey or calf. Not only was the relative percentage of *darlingi* higher when the trap was baited with man, but the actual number of *A. darlingi* females caught in this trap was higher than in the traps baited with donkey and calf, in spite of the fact that the total mosquito catch was much lower when man was used as bait. The experiments

were carried out under such parallel conditions that the results leave no doubt as to man's greater attractiveness to *A. darlingi* than that of the domestic animals used in these trials. If the results of house captures and of stable trap captures with man as bait are compared (Table X), it can be seen that the distribution of anophelines is practically the same in both cases, and this leads us to the conclusion that it is the presence of man in the dwellings which makes them attractive to *A. darlingi*.

If the results of *darlingi* are compared to those of *argyritarsis*, it will be observed that the last species is, on the contrary, more attracted by the donkey and calf than by man, thus explaining its small numbers in house captures, in spite of its abundance in the savanna.

We had noticed that the inhabitants of the Llanos usually clear the grass around their dwellings, leaving the ground bare for 5 to 30 m. around the house. This is incorrectly called by the local people, a "patio" or courtyard, and it is a characteristic feature of the typical "Llanero" dwelling. The local people believe that this traditional practice keeps the houses fairly free from mosquitoes and gnats, and we found that if a stable trap is set up within the cleared area and another away in the grass, but at no more than 10 m. from the first one, the mosquito catch in the grass is several times higher than that in the cleared area.

We observed in a camp of the Shell Co. in the Llanos that, though mosquito density was high in the neighboring savanna, no mosquitoes were caught within the 100 m. radius of clearing made with bulldozers around the buildings. It should be noticed that these observations were made in the Llanos proper with their characteristic mosquito population of *A. pessoai*, *A. peryassui* and *A. parvus*. In the San Antonio study area, the clearings made around the houses by the newcomers are usually small, no more than 5 m. wide round the houses, but we decided to try whether clearings had any effect on the local mosquito fauna. For that purpose a circular clearing of 30 m. radius was made around one of our stable traps, the other being left at 100 m. distance in the untouched savanna. The traps were baited with two donkeys which were exchanged every night to avoid a possible difference in attractiveness. To prevent the grass growing in the cleared area, the new shoots were rooted out every week. Captures were made on 28 consecutive nights and a total of 10,220 specimens were collected, their distribution being shown in Table IV.

As can be seen, the total mosquito catch in the control area was more than 3 times as large as the catch in the cleared area. The clearing thus produced a marked diminution in mosquito numbers, confirming our previous observations on clearings (de Zulueta, 1950a). But, as can also be seen, the clearings had no effect on *A. darlingi;* in fact there were more females of this species caught in the cleared area than in the control, which seems to indicate that the blood seeking females of *darlingi* flying over the grass are able to spot a bait placed 30 m. away, whereas the females of *pessoai* met with difficulties and the majority of *argyritarsis* females failed to detect the bait in the cleared area. This indicates that the clearing of grass may be a useful measure for mosquito control in a savanna country when the anopheline species are other than *A. darlingi;* but it

shows also that small clearings can have little effect on malaria control in those areas where *A. darlingi* is the chief vector.

Captures of resting mosquitoes

These captures were made during the day-time between 7 A.M. and 5 P.M. using the portable cage already described. The results are summarized in Table VII. As can be seen, the findings are divided under two headings: captures made in the savanna within 100 m. radius of the dwellings used as capture stations and catches made in the open savanna away from houses and in the vicinity of the site of the stable traps, at a distance of roughly 500 m. from the dwellings.

The results summarized in Table VII show, first of all, that the average mosquito density was lower than that previously reported by us. But it must be noticed that the bulk of the captures presented here were made in 1950. The

TABLE VII

Results of 732 catches of resting mosquitoes made in the daytime; (based on 2,616 specimens)

	OPEN SAVANNA AWAY FROM HOUSES	SAVANNA, WITHIN 100 M. RADIUS OF HUMAN DWELLINGS
Number of trials	496	236
Male anophelines captured	78	28
Female anophelines captured	65	49
Male culicines captured	930	480
Female culicines captured	662	324
Total mosquitoes captured	1,735	881
Anophelines per sq. m.	0.09	0.10
Culicines per sq. m.	1.04	1.09
Total mosquitoes per sq. m.	1.13	1.19
Engorged anopheline females (per cent)	40.0	68.8
Engorged culicine females (per cent)	24.8	31.9

average density found in the 70 captures made in this area in November '49 was 3.1 mosquitoes per sq. m., roughly the same density observed in 1948 and '49 in our first report on this subject. The lower mosquito density observed here must therefore be attributed to a diminution in the mosquito populations in 1950 compared with the previous years.

It will be observed also from the results of Table VII that the mosquito density was approximately the same near the houses as far away from them; in both cases the culicines formed the bulk of the captures, and the anophelines only about a tenth, which again confirms our previous findings on mosquito density in a savanna country. However, our results relating to the number of engorged females differ from what we found previously in uninhabited areas. We find here that 40.0 per cent of the anopheline females caught in the open savanna were engorged and 68.8 in the vicinity of houses; in the case of the culicines the corresponding figures are 24.8 and 31.9. This shows that the expectation of a mosquito finding a blood meal is higher in an inhabited area like the San An-

tonio settlement than in savannas devoid of human population and domestic animals, where we found that only 11 per cent of the females were engorged; also that the chances of finding a blood meal are higher for the anophelines than for the culicines, and finally that a human dwelling provides a more profitable source of blood than the domestic and wild animals scattered in the open savanna.

TABLE VIII

Results of 11 captures of flying mosquitoes made between 6:10 and 6:30 P.M.; (based on 1,582 specimens)

	MALES	FEMALES	TOTAL
Culicines	804	499	1,303
Anophelines	215	64	279

TABLE IX

Distribution of culicines and anophelines in the various types of captures made

TYPE OF CAPTURE	PER CENT DISTRIBUTION	
	Culicines	Anophelines
House captures	2.1	97.9
Stable trap catches with man as bait	18.1	81.9
Stable trap catches with donkey as bait	31.1	68.9
Stable trap catches with calf as bait	78.9	21.1
Captures of resting mosquitoes	91.6	8.4
Captures of flying mosquitoes	82.4	17.6

TABLE X

Distribution of anopheline species in the various types of captures made

TYPE OF CAPTURE	ANOPHELINE SPECIES—PER CENT DISTRIBUTION			
	A. pessoai	*A. darlingi*	*A. argyritarsis*	Miscellaneous
House captures	27.5	70.0	0.8	1.7
Stable trap baited with man	24.7	72.0	2.5	0.8
Stable trap baited with donkey	40.5	19.7	37.6	2.2
Stable trap baited with calf	56.1	6.7	31.6	5.6
Captures of resting mosquitoes	67.7	0.5	25.9	5.9
Captures of flying mosquitoes	77.9	1.9	9.2	11.0

A relatively higher number of anopheline females was found in the vicinity of houses than away from them. The sample in this case is small but the finding nevertheless seems to indicate that the anopheline females coming in contact with human dwellings tend to rest in their vicinity.

We counted as engorged females those which showed under the microscope a stomach containing blood, or had developing ovaries. The results of the captures of resting mosquitoes show also that the number of males was higher in the case of the culicines than the number of females and approximately the same in the

case of the anophelines. This confirms our previous results (1) and seems to show that the survival in nature of both sexes is probably the same and that the assumption of a shorter span of life for the males, drawn from laboratory observations, is probably incorrect.

The list of the anophelines found resting in the grass in the San Antonio study area is worth presenting:

	MALES	FEMALES
A. pessoai	57	92
A. argyritarsis	43	14
A. peryassui	6	4
A. benarrochi	0	2
A. darlingi	0	1
A. albitarsis	0	1
Total	106	114

As can be seen, *A. pessoai* was found more frequently than all the other species put together. The only specimen of *A. darlingi* collected was an engorged female found in the savanna near the forest edge and far away from houses. If these captures reflect the relative sizes of the savanna populations, it will be seen how small is the actual population of *A. darlingi*.

Captures of flying mosquitoes

They were made according to the techniques described above. The average results of 11 captures made with the nets set at 1.5 and 4.5 m. above the ground, are summarized in Table VIII. It will be seen that males as a whole were more abundant than females, but this may be due in this case to a greater activity of the males after sunset, rather than to a really greater number. Though these captures can be affected by the hour at which they are made and by the height at which the nets are set, the fact that only 17.6% of the mosquitoes were anophelines (Table IX) again seems to show that the anopheline population is much smaller than the culicine. The identification of a sample of anophelines caught with the nets gave the following results:

A. pessoai	120
A. argyritarsis	14
A. peryassui	9
A. darlingi	3
A. parvus	3
Miscellaneous	5
Total	154

We can see again from these results that *A. darlingi* forms in fact a very small fraction of the local anopheline population, a fact which would not be suspected from the results of the house captures.

Before concluding this part, we may add that the anophelines coming under the heading of "miscellaneous" in the text and tables belong to one of the following species: *A. oswaldoi*, *A. benarrochi*, *A. albitarsis*, *A. parvus*, *A. peryassui*, *A. rangeli*, *A. triannulatus*, *A. punctimacula* and *A. mediopunctatus*. Common culicine species found in the course of our work in this area were *Culex* (*Melanoconion*) *mistura*, *Culex* (*Melanoconion*) *rooti*, *Uranotaenia pulcherrima* and *Aedes* (*Ochlerotatus*) *eucephalaeus*.

DISCUSSION

The findings reported in this paper show a well marked difference between the results obtained from often employed methods such as domestic captures and stable trap catches and the results of more direct methods of estimating the mosquito density and the species composition in a given area. Certain species came in large numbers to the houses, attracted by man, whereas other species, though very abundant in the surrounding savanna, were hardly brought in contact with the human dwellings. This leads us to the discussion of the host preference of mosquitoes. Since the early studies of the maculipennis "complex" in Europe, the terms "anthropophilous" and "zoophilous" have been widely used in current mosquito literature, but, as Bates has pointed out (1949), "the distinction, from a biological point of view, seems unfortunate, since man is thus singled out in a dichotomy opposed to all the rest of the animal kingdom". The term is no doubt inadequate in spite of its wide use but the fact remains that certain mosquito species may show a definite preference for man. In the experiments reported in this paper, *A. darlingi* clearly preferred man to calf or donkey, when it could choose between them under identical conditions. *A. argyritarsis*, on the other hand, prefered donkey or calf to man in the same experiments. *A. darlingi* could thus be labelled with the widely but inaccurately used term of "anthropophilic" and *A. argyritarsis* as "zoophilic".

We plan to discuss in a separate publication the bionomics of *A. darlingi*, the chief malaria vector of this region, but it is worth emphasizing here the fact that this species was easily captured in houses during the day-time in Venezuela (Gabaldón, 1949) and British Guiana (Giglioli, 1948) prior to DDT spraying, whereas, as we have mentioned before, except for the females caught in the mosquito nets, no specimen could be found during the day-time in the houses of San Antonio or in any other place studied in Eastern Colombia. Besides, as Benarroch (1950) and Gabaldón (1949) state, ordinary stable traps are inadequate for catching this mosquito in Venezuela. We ourselves found in the Villavicencio area (Bates *et al.*, 1949) and in the course of the present observations, that this species, though more abundant in houses during the night, can be caught in stable traps with animal bait. Such marked differences in the bionomics of *A. darlingi* have a considerable importance not only from the biological point of view but also from that of malaria control. *A. darlingi* seems here to be less associated with man and less in contact with human dwellings than in other places where malaria control by DDT house spraying has met with complete success.

The results summarized in the present paper confirm our former observations that, in the savannas of this region, the clearing of grass has a marked and lasting effect on mosquito numbers. This effect was mainly noted in the case of *A. argyritarsis*, was less marked with *A. pessoai* and in the case of *A. darlingi* no effect could be detected with a clearing of 30 m. radius. Hackett *et al.* (1938) in a review of naturalistic measures for the control of malaria, stated that no effect had been observed, to date, from clearings of vegetation. Ribbands (1946), summarizing his more recent work in Africa, makes clear that bush clearance had no effect on the anophelines coming to bite. The experiments were carried out in an area of *A. gambiae* and *A. funestus*; only while the clearing was in progress was a diminution of the numbers noted, to be followed a week after the clearing was finished by a rise to the numbers found previously.

The method of catching mosquitoes with a tent or with a portable cage, as in the experiments reported above, is again recommended as a simple and direct method of estimating the real size of mosquito populations. The results of the captures referred to in this paper confirm our first impression (1) that this simple method gives more direct information about the mosquito density and species composition in an area of savanna than any other method tried. Estimation of the real mosquito density in a given area was tried for the first time by Eyles and Cox (1943) with *A. quadrimaculatus*. The population of anopheline imagines was measured by an indirect method; large numbers of resting mosquitoes were caught, marked with bronze dust and released, to be again recaptured. From the data gathered, the total population could be mathematically estimated and it was found that the density of *A. quadrimaculatus* in the study area was remarkably high. The method we have described has the advantage of its simplicity and of giving information on all the mosquito species dwelling in a savanna country.

This technique is complemented by catches of mosquitoes on the wing by means of nets fixed to a moving vehicle. The results in both cases are very similar, in spite of the fact that the captures of flying mosquitoes must be affected by the time of their activity. We have found that a method based on the same principles was tried with good results by Chamberlin and Lawson (1945).

SUMMARY

The observations reported in this paper were made in a small settlement which exemplifies the conditions met with in an endemic malarious area in the savanna country of Eastern Colombia. The studies were aimed at obtaining comparative information about the mosquito density in the savannas and the samples obtained in house captures. Direct information on mosquito density was obtained by means of daytime captures of resting mosquitoes made with a portable cage and with evening captures of flying mosquitoes made with nets fixed to a moving vehicle.

The results of such captures were widely different from those obtained from house captures. It was proved that this was mainly due to host preference and,

to a certain extent, to the clearing of grass around the houses. Of the anopheline mosquitoes found in the area, *A. darlingi* showed a clear preference for man when given the choice, under identical conditions, between man and domestic animals such as donkey and calf. *A. darlingi* proved to be more able to detect a bait at a distance than the other anophelines, thus rendering the small clearings round the houses ineffective against this species. *A. argyritarsis* behaved in the opposite way, being little attracted by man and easily deterred by grass clearance. *A. pessoai* showed an intermediate behavior between these two species.

Measuring the mosquito density in the savannas by the direct methods mentioned above, which are not influenced by any host preference, it was found that, of the anophelines present in the area, *A. pessoai* was by far the most abundant. *A. argyritarsis* had a much higher density than the house captures would lead one suspect. *A. darlingi*, on the other hand, proved to have a very low density, utterly disproportionate to the considerable numbers caught in human dwellings.

As in previous observations, we found that the culicines were about ten times more abundant in the savannas than the anophelines. They were little attracted by man and hardly entered the human dwellings.

Mosquito density within a 100 m. radius of houses was approximately the same as in the open savanna at half a kilometre from any human dwelling, but a relatively higher number of anopeheline females was caught near the houses than away from them. Though the sample in this case is small, this seems to indicate that the anophelines brought into contact with the human dwellings tend to remain in their neighbourhood.

Engorged females of both anophelines and culicines were more abundant in the vicinity of houses than away from them. The average number of engorged females found in these experiments was several times higher than the average number found previously in uninhabited areas.

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THE FACULTATIVE BREEDING OF *HAEMAGOGUS SPEGAZZINII FALCO* KUMM *ET AL.*, THE VECTOR OF JUNGLE YELLOW FEVER IN COLOMBIA

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Haemagogus spegazzinii falco Kumm *et al.* (1946) is the proved vector of jungle yellow fever in the endemic region around Villavicencio, in the Intendencia of Meta, Colombia. This species has been found naturally infected with yellow fever virus on many occasions. Bates (1949) states that this species and the closely related *H. spegazzinii* Brethes have been found infected at least 17 different times in Colombia and Brazil.

Several observers have noted the difficulty in finding the larval breeding places of this and closely related species in numbers sufficient to account for the swarms of adults which can be captured on elevated platforms in the forest. Causey and Dos Santos (1949) mention the scarcity of larvae of *H. spegazzinii* in the region around Passos, in Minas Geraes, Brazil. Bugher *et al.* (1944) state: "larvae (of *H. falco*) in nature are found almost entirely in tree-holes, but this finding is irregular and the number found does not always seem commensurate with the population of adult mosquitoes."

During my visit to the Rockefeller Foundation Laboratory at Villavicencio, Colombia, in the spring of 1944, Dr. Marston Bates, who was in charge, mentioned the difficulty in finding *Haemagogus* larvae, and we decided to try to find the larval breeding places. In this attempt, the combined knowledge of two experienced mosquito entomologists was brought to bear, and we sampled every known or probable larval habitat in and surrounding the Forzosa forest area (Bates, 1944), near Villavicencio. We searched the area for at least a week. Several tree-holes were discovered in this high forest, which were at least fifty feet from the ground. The trees were felled and the holes examined, but only larvae of an *Orthopodomyia* species were found. A survey of accessible tree-holes in the neighborhood of Villavicencio had been instituted some time previously in which Colombian helpers examined the tree-holes at regular intervals, brought back to the laboratory any larvae found in them, and replaced the water removed. Our own extended search was negative, nor were *Haemagogus* larvae found in the regular tree-hole survey. Yet during May, June, and July, the months of greatest abundance of *Haemagogus* adults in the forests (Bates, 1945), these "were numerous at the tree-platform collecting stations, and were caught biting at ground level at midday in the forest."

In contrast to the usual difficulty in finding larvae of *Haemagogus falco* is the writer's experience in a region close to Villavicencio during his first visit to Colombia in the early summer of 1935. The yellow fever laboratory of the Rockefeller Foundation at that time was in the small town of Restrepo, some 20 kilometers north of Villavicencio. (For maps of the region, see Bugher *et al.* (1944).) Just before entering the town on the mule trail from Villavicencio is a suburb known

locally as Balconcito. This is a small area of second-growth forest from which the townspeople obtained firewood. Many stumps and fallen trees were present, and a hairy larva of *Haemagogus* was found in large numbers in the water in holes in these stumps and trees, in locations accessible to the collector standing on the ground. This species was identified by the writer (1936) as *Haemagogus janthinomys* Dyar, which is a synonym of *H. spegazzinii* Brethes. It is now known to be *H. spegazzinii falco* Kumm *et al.* The writer well remembers collecting many larvae of *H. falco* from the water-filled hollow formed by two buttresses of a large fallen tree, lying alone in full sunlight in a field near this area. Larvae were also found in a depression at ground level, formed by the rotting out of a large palm stump, which had left a conical cavity filled with dark brown water. On many other occasions larvae were found in the water in cut stems of bamboo, usually along trails leading to small farms. These large bamboos had been cut for building material and were in the neighborhood of human habitations. After the initial fermentation of the starchy matter in the lining of the internodes, they were suitable for the larvae of *H. falco*, and these were taken at ground level in many collections.

Some years later, the larvae of *H. spegazzinii* Brethes were collected by the writer from cut bamboo at ground level under similar conditions, at St. Ann's, a suburb of Port of Spain, Trinidad, B. W. I. Here conditions were very like those found near Restrepo, in that there was an apparent scarcity of other suitable breeding places, and human blood was readily available.

From these observations it would appear that the breeding of *H. falco* may be facultative. That is, if the situation is made favorable for the breeding of the larvae by artificially producing tree-holes, cut bamboo, fallen tree-buttresses holding water, and other similar larval habitats, and by providing an abundance of human blood, this species will take advantage of these abnormal conditions. Larvae will be found in habitats which do not normally occur in high rainforest, where presumably the natural breeding places should be found. These are not at the present time definitely known.

Conditions similar to those in the Balconcito suburb must be common to many small towns in Colombia and Brazil. Such situations may be potentially very dangerous with respect to the transmission of sylvan yellow fever. The abundance of *Haemagogus* adults, particularly at ground level in coffee plantations in the Andean foothills, as noted by Bugher *et al.* (1944), would afford ideal conditions for infection with yellow fever. The usual protective method against yellow fever is vaccination of the exposed population. This was not very effective in the endemic area around Restrepo, at least in 1944. The region was revisited in the spring of that year with Dr. Bates. A portable sawmill had been set up in the Balconcito suburb, and the sawyers were living in tents near the mill in the forest. They were thus in a very vulnerable position with regard to infection with yellow fever because of the relatively large numbers of *Haemagogus* present at ground level, and because they were felling trees, thus bringing the *Haemagogus* mosquitoes, normally inhabitants of the forest canopy, down to the ground. None of these men had been vaccinated against yellow fever.

The peculiar conditions existing in the Balconcito area were very favorable for the facultative breeding of *H. faico*, for the reasons stated. They were quite in contrast to the probably more normal conditions for the breeding of this species in the high Forzosa forest, where no *Haemagogus* larvae were found during the writer's search.

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HOUSE FLIES IN EGYPT

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The taxonomy of flies of the genus *Musca*, and particularly of the important and widespread species or forms of the "*domestica* complex," has received considerable attention in many parts of the world. In spite of this, the status of such forms as *Musca vicina* Macq., *M. nebulo* F., and *M. cuthbertsoni* Patton is not at all clear. Consequently, it is worthy of note that the work now being carried on at the laboratory of the U. S. Naval Medical Research Unit (NAMRU-3) in Cairo, Egypt, and the opportunities there for coordinated field and laboratory studies of several of the forms and their interactions, offer real hope for securing significant information on the problem.

During a six weeks' assignment at NAMRU-3 in June and July, 1950, as consultant on taxonomic problems in the genus *Musca*, I determined that at least eleven species and subspecies of *Musca* occur in Lower Egypt, as follows: *domestica vicina* Macq., *domestica cuthbertsoni* (tentatively regarded as a subspecies), *sorbens sorbens* Wied., *crassirostris* Stein, *xanthomelas* Wied., *tempestiva* Fallén, *albina albina* Wied., *vitripennis* Meigen, *larvipara* Portsch., *sorbens alba* Mall., and *lucidula* Loew. The status of *Musca domestica domestica* L. and *M. domestica nebulo* F., which have been recorded from Egypt, is discussed later.

At the time of my study, *vicina* was by far the dominant fly of the metropolitan areas of Cairo and Alexandria, and it was the common fly of most of the Delta villages from which samples were available. *Cuthbertsoni* was predominant in a few places, common in a few, and occurred occasionally in others. *Sorbens* was collected in many places, although it did not appear to be abundant anywhere, but its habits are so unlike the gregarious and domestic *vicina* and *cuthbertsoni* that ordinary methods of collecting undoubtedly do not give a fair idea of the number of individuals per unit area.

Most of the other species were infrequently or rarely found. However, extensive collecting in the desert and isolated oases would probably have produced a greater number of the less common species. For example, at the small oasis at Wadi Natroun between Cairo and Alexandria, six species of *Musca* were collected in a small irrigated garden plot only a few feet square. Two-thirds of the speci-

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mens were *albina*, *tempestiva* and *xanthomelas*, three species which were only rarely observed elsewhere. In contrast, all but one of the flies collected inside houses at Wadi Natroun were *vicina* or *cuthbertsoni*.

Adult habits of the species differ markedly, and this may well be of considerable significance in relation to disease transmission and to control. The forms of the "*domestica* complex" *vicina* and *cuthbertsoni*, are characteristically domestic, and they occur gregariously in considerable numbers in cafés, bazaars, abattoirs, food stores, and houses. *Musca sorbens* is sometimes present in all of the above places, but in small numbers, though again the method of collecting may not have been the most effective for that species. In my experience, *sorbens* was more often directly annoying to humans than either *vicina* or *cuthbertsoni*. In Cairo, about two-thirds of the flies that were collected while annoying me were *sorbens*, even though general Cairo collections were overwhelmingly *vicina* and *cuthbertsoni*. At Oasis Feiran in Central Sinai, where *sorbens* was the predominant species, it was a particular nuisance to sleeping campers at daybreak. This predilection for humans should be investigated further as it may be indicative of special significance in the epidemiology and transmission of disease.

Identification of lots of flies reared by R. L. Peffly in March and April 1950 from various materials collected at a village in Giza province near Cairo provided some interesting leads that should be investigated further, particularly in other areas and at other seasons. In five different lots of over a hundred specimens each, reared from animal rooms, compost heaps, and latrines, there was a total of 815 *vicina*, 20 *cuthbertsoni*, 9 *sorbens*, and 53 *Sarcophaga* spp. In contrast, three samples reared from human stools had a total of 1 *vicina*, 833 *sorbens*, and 386 *Sarcophaga* spp. The samples had 219, 211, and 403 *sorbens*, respectively. These observations suggest that diminution or even control of the *sorbens* nuisance might be obtained through improved disposition of human fecal matter, independent of the use of insecticides. If so, it would be interesting to observe whether there would be concomitant effects on the prevalence of eye diseases and various sores, inasmuch as *sorbens* is so commonly found crawling on the faces.

Fuel cakes have been suspected of producing numerous house flies, but eight samples from the above village were found to contain a total of only 29 *vicina* and four specimens of three uncommon species of *Musca*, together with 386 *Paregle cinerella* (Fallén) (a small muscid fly related to the great genus of root maggots, *Hylemya*), and 13 specimens of two minor species of other muscid genera. In the general problem of fly control, it will obviously be important to determine if this low level of production of *Musca* is true for fuel cakes in general throughout the year.

Special attention was given to a study of variation in taxonomic characters of the genus *Musca*, especially in connection with the classification of *Musca domestica* and its several forms. The excellent insectary facilities and accessible material made it possible to carry on carefully segregated rearings in order to observe variation in the progeny of single females, though this work was naturally restricted in scope and number of observations by the limited time available.

The principal morphological characters that have been used for distinguishing

forms of the *domestica* complex are the length of the postsutural dorsocentral bristles and, in the males, the width of the front between the eyes. In general terms, typical *domestica* has long bristles and broad front, *vicina* long bristles and a front of intermediate width, *nebulo* long bristles and narrow front, and *cuthbertsoni* short anterior postsuturals and narrow front. Typical *domestica* was not available in Cairo, though series from the United States and northern Europe were later measured for comparison.

Examination of series reared from eggs laid by isolated females brought out the following:

(1) Long-bristled females (*vicina*) consistently produced long-bristled offspring.

(2) Short-bristled females (*cuthbertsoni*) had progeny that ranged from very short-bristled individuals, in which the two anterior postsutural dorsocentrals were but little longer and stronger than hairs, to some in which those bristles appeared as long as in *vicina*. The length of the first bristle in one long series of siblings ranged from 0.20 to 0.68 times the length of the fourth or hindmost postsutural bristle.

(3) The great range in length of the posterior dorsocentral bristles in series of siblings demonstrates conclusively that bristle length cannot be used as an absolute criterion in identifying any single specimen. This may still not invalidate the character, of course, as a useful means of recognizing most specimens of *cuthbertsoni*, if the latter does prove to be a distinct form.

(4) Progeny of *vicina* and *cuthbertsoni* overlap [illegible]atly in certain combinations of characters. Specimens of the former with narrower front than usual are virtually indistinguishable from those occasional specimens of the latter which have long bristles and a wider front than usual. However, there may still be two distinct populations in nature, and the normal curves of distribution for characters of the two may overlap for only a small percentage of individuals. Coordinated study of geographical and seasonal distribution may also furnish evidence that *cuthbertsoni* is a natural population distinct from that of *vicina*. In this connection, it may be significant to note that *cuthbertsoni* has thus far been regarded as typically a species of the Ethiopian Region. Furthermore, the character of short dorsocentrals has not been observed to my knowledge in any populations of the "*domestica* complex" in temperate regions or in the Western Hemisphere.

(5) In the offspring of *cuthbertsoni* females, an occasional male showed the combination of narrow front and long dorsocentral bristles. These specimens, if found on pins in museum collections, would have been identified as *Musca nebulo* (or *M. domestica nebulo*). This form was recorded from Egypt by Patton (1933, Ann. Trop. Med. and Parasitol. 27: 341, 401–403), though he later (1937, loc. cit. 31:129) expressed some doubt as to whether the Egyptian form was the true *nebulo*, which had originally been described from the Orient. My results suggest that *Musca nebulo* as identified from Egypt does not represent a population and thus neither deserves nor requires a name. The status of the oriental *nebulo* is another problem, which may or may not be affected by these findings.

No populations of *Musca domestica domestica* were found in Egypt, although

two males with the habitus of typical *domestica* were collected at Alexandria. However, progeny of females of *vicina* contained occasional males with the characteristically broad front of typical *domestica*. Accordingly, the findings of occasional individuals cannot be considered as necessarily indicating the presence of either individuals or populations of typical *domestica* in Egypt. It is possible that earlier records of it were based on such variant examples of *vicina*, though of course it is also possible that the records were based on true *domestica* introduced from southern Europe. The finding of atypical individuals recalls that specimens with a narrow, *vicina*-like front are sometimes found in temperate zone populations of typical *domestica*, but they are recognized as mere variants.

The information resulting from the above studies is only a preliminary step in the perplexing problem of *Musca domestica* and related forms. Laboratory studies of *vicina* and *cuthbertsoni*, on the range of variation in taxonomic characters, their Mendelian inheritance, crossbreeding, and other phases, are needed as essential background for solving the problem of the status of the two forms. Along with these studies should go a thorough field study of the interaction of populations of these forms in nature. The various species or subspecies are involved in investigations of disease transmission and resistance to insecticides in many parts of the world, and it is of great practical importance to clarify their status. The laboratory of NAMRU-3 at Cairo is strategically located and excellently equipped for making significant contributions on this long-disputed problem.

THE COMMON HOUSE ROACH, *BLATTELLA GERMANICA* LINN., AS A POTENTIAL VECTOR OF *SALMONELLA TYPHIMURIUM* AND *SALMONELLA TYPHOSA*

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The cockroach has long been suspected of harboring and disseminating disease organisms. Relatively little work, however, has been done definitely to implicate these insects as vectors of disease. The filthy habits and cursorial nature of roaches make them ideally suited for the mechanical spread of certain pathogenic organisms by contamination of foodstuffs and fomites through contact with the infected appendages of the roach (Steinhaus, 1947; Fox, 1925; Longfellow, 1913; Herms *et al.*, 1913).

The omnivorous feeding habits and indiscriminate deposition of fecal material by the roach presents the additional possibility that the alimentary canal of these insects may serve as a reservoir for multiplication of certain pathogens followed by subsequent excretion with the fecal matter. This latter possibility was the stimulus for the research here presented.

That the cockroach may be infected with pathogens while in its natural environment has been demonstrated by a number of workers. Mackerras and Mackerras (1948) were able to isolate *Salmonella bovis-morbificans* from the intestinal contents of a number of *Periplaneta americana* captured in a hospital ward where an outbreak of salmonellosis was occurring. Recently Bitter and Williams (1949) reported the isolation of *Salmonella schottmulleri*, *Salmonella oranienburg*, and *Salmonella bredeney* from the hindgut contents of *Periplaneta americana* captured in San Antonio, Texas.

Longfellow (1913), Macfie (1938), Olsen and Rueger (1951), Mackerras and Pope (1948), Wedberg, Brandt and Helmboldt (1949) and other workers by experimentally infecting various species of cockroaches have demonstrated that a number of different bacteria can be made to pass through the alimentary canal of the roach without loss of viability. There is sufficient direct and indirect evidence to warrant further study of the cockroach as a vector of disease and to initiate stronger measures designed to exterminate this insect, especially in hospitals and in public eating establishments.

Blattella germanica, called the Croton Bug or German Roach, was selected for this study since it is widespread and is very commonly found in close association with the food and refuse of man. The normal habits of this roach are ideally suited for the transmission of enteric pathogens such as *Salmonella* species. Controlled feeding of the roaches with cultures of *S. typhimurium* and *S. typhosa* was undertaken based on the premise that if the intestines of roaches could be implanted under controlled conditions, and viable salmonellas recovered from the excreta or the alimentary tract, the insects could conceivably become infected under natural conditions and serve as vectors of salmonellosis. Although

controlled experiments merely cast suspicion on the insect, as opposed to the more convincing and direct evidence provided by isolation of pathogens from insects captured in their natural environment, the former method of study was chosen since the duration of roach infectivity could best be determined by this technique.

METHODS AND MATERIALS

All of the roaches studied in these experiments were captured in one of the buildings at the University of Connecticut. The stock colony of unmounted insects was supplied a diet of Pablum and water, and only adult roaches were chosen for study. Female roaches were employed in larger numbers than male roaches.

A modification of the technique described by Wedberg and Clarke (1947) was used in mounting the individual roaches on a thick paraffin film attached to the flattened end of a glass rod. The roach was first inactivated by placing it in a test tube and exposing it to a temperature of 5–10°C. for five minutes. The paraffin film was melted with a heated needle and the chilled insect was placed with the dorsum in contact with the paraffin. As soon as the roach was firmly attached, the hind legs were imbedded in the paraffin to minimize mechanical contamination of the droppings.

Each mounted roach was suspended within a sterile 250 ml. widemouth bottle containing 3.0 ml. of sterile saline in which the feces were allowed to collect. A dust-proof air conduit was provided which could be removed and replaced with a potometer consisting of a 0.1 ml. serological pipette with a modified feeding tip through which liquid nutrients could be fed and the amount of food consumed accurately observed.

Individual insects were fed an exclusive diet of molar sucrose solution, beef heart infusion broth, or skim milk to which had been added 1.0 per cent yeast extract and 0.5 per cent sucrose. The three individual diets were tubed in small amounts and were autoclaved for ten minutes at 15 pounds pressure (120–121°C.). A tube of nutrient solution was used for only one feeding to minimize contamination. Three different diets were employed to observe their effect, if any, on the passage of the test organisms through *Blatella germanica* and on the longevity of the confined roaches.

A strain of *S. typhimurium* isolated by Wedberg during a mass food infection caused by lemon pie at Camp Edwards, Massachusetts in the summer of 1944, and a strain of *S. typhosa* (Watson IV) originally received from P. R. Edwards of the University of Kentucky, were used in the controlled feeding experiments. Growth from 24 hour standard agar slant cultures of the organisms was suspended in sterile saline and adjusted to a turbidity of 6.0 on the McFarland Nephelometer scale. Plate counts of the suspensions revealed approximately 1×10^9 organisms per ml. Each roach was fed between 0.005 and 0.010 ml. of this standardized suspension, and all feedings were carried out in sterile bottles other than those in which the droppings were to be collected. After each feeding the insects were kept in the feeding chamber for at least one hour before being returned to the

stool-collecting chamber. This practice helped to minimize possible contamination of the saline from regurgitation of the roach or from infected droplets clinging to the appendages. Regurgitation was observed only during the first few minutes following too generous a feeding and this undesirable feature was effectively controlled by limiting the quantity of solution per feeding to the average daily intake of the individual insect.

The saline tubes were inspected at least once every 18 hours and any feces collected during this period were immediately emulsified in the saline and the roach removed to a new sterile chamber. A loopful of the fecal emulsion was streaked on Difco SS agar, and the remainder of the emulsion was pipetted to a large tube of Selenite-F broth which was incubated for 18 hours at 37°C. The incubated broth was streaked on Endo agar which in turn was incubated for about 18 hours at 37°C. Any colonies characteristic of the *Salmonella* group were transferred to Difco triple-sugar iron agar slants. Organisms exhibiting typical *Salmonella* reactions on the triple-sugar iron agar were subcultured in suitable media for physiological confirmation.

When the alimentary canal contents of a roach were to be studied, the insect's head and appendages were burned off with a heated needle, and the torso was immersed in 1:1000 tincture of merthiolate for five minutes and then allowed to dry in a sterile petri dish. The alimentary canal was removed from the roach and macerated in Selenite-F broth. Streak platings were made from this broth on Endo agar and on SS agar both before and after incubation of the Selenite-F. Confirmation of positive cultures was carried out using the same methods as those employed with the fecal emulsions.

LONGEVITY STUDIES

The first findings here presented were designed to determine the effect of the mounting technique, confinement, diet, and the feeding of specific salmonellas on the longevity of *Blattella germanica*.

Roaches which had been placed in test tubes and starved to death survived no longer than starved roaches mounted on the paraffin blocks; survival averaged about ten days in each case. The feeding of the *Salmonella* cultures had no significant effect on longevity of the insects when compared with control roaches. There was considerable variation, however, in survival with respect to the diet fed to the roaches. Beef heart infusion broth proved to be the poorest of the three diets tested from all standpoints considered. While the fortified skim milk diet seemed to be ingested in larger volume and resulted in apparently larger droppings, the molar sucrose solution kept the roaches alive for longer periods of time. In one instance a female roach lived for 205 days on an exclusive diet of molar sucrose. She produced two egg cases during this period and 29 young hatched from one of these cases. This is confirmation of an observation of Trager (1941) who stated that "Numerous species of insects can live in the adult stage and reproduce without taking any food at all. Others require only water, others carbohydrates, while others can live on a carbohydrate diet but must have other nutrients in order to reproduce".

BACTERIAL FLORA OF THE ALIMENTARY CANAL

Studies of the excreta and alimentary canal contents of 13 control roaches were undertaken to ascertain the common types of aerobic and facultative bacteria which might conceivably be confused with the test organisms being fed to the *Blattella germanica*.

Although gram negative rods predominated, no single species was found to be common to all of the roaches studied. *Alcaligenes* and *Achromobacter* were most common in roaches fed the beef heart infusion broth, while a yeast belonging to the *Saccharomyces* genus was consistently found in roaches fed molar sucrose solutions.

The following organisms were isolated from the sources under consideration and were identified according to the sixth edition of *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1948): *Achromobacter* spp., *Aerobacter cloacae*, *Alcaligenes fecalis*, *Alcaligenes viscosus*, *Bacillus circulans*, *Escherichia freundii*, *Micrococcus aurantiacus*, *Micrococcus epidermidis*, *Micrococcus pyogenes* var. *albus*, *Micrococcus ureae*, *Paracolobactrum aerogenoides*, *Paracolobactrum coliforme*, *Pseudomonas aeruginosa*, and *Streptococcus* spp. The fact that these organisms were isolated from the feces and the alimentary canal of *Blattella germanica* indicates that they are part of the potential normal flora, but the omnivorous feeding habits of this roach would make it very difficult to state which bacteria constitute the so-called normal flora. No anaerobic determinations were conducted.

CONTROLLED FEEDING OF *S. typhimurium* AND *S. typhosa*.

This phase of the investigation dealt with attempts to pass a specific *Salmonella* species through the alimentary canal of *Blattella germanica*. Each roach was checked before feeding the test organisms to be certain that no bacteria resembling salmonellas were present to give false positive findings. Each roach was fed with the aid of a potometer at least 0.005 ml. of a broth culture of the test organisms, and droppings were collected, counted, and cultured as previously described. The results of these studies are shown in table 1. The duration of stool infectivity was determined and the presence of salmonellas in the alimentary canal contents was noted after sacrificing the roaches on the days indicated.

Using techniques similar to those employed with the *S. typhimurium* cultures, 21 individual roaches (17 females and 4 males) were given a single feeding of a *S. typhosa* broth culture containing between 3×10^6 and 3.8×10^7 organisms. As many as seven stools were collected from a single roach over an 11 day period, but in only 2 instances did cultures of these passed stools reveal the presence of the test organisms. Both of these stools were passed within 18 hours after the organisms were fed to the roaches. The basic diet in one instance was brain heart infusion broth and in the other case fortified skim milk. Only one alimentary canal was positive for *S. typhosa* and this was on the second day after the culture had been fed to the roach. Chance contamination cannot be overlooked. Wedberg, Brandt, and Helmboldt (1949) reported negative findings in their attempt to pass this organism through *Blaberus cranifer*.

Examination of the saline in the bottles containing the experimental roaches which had not defecated during a given 18 hour period was carried out to be certain that no salmonellas were finding their way into the saline except through the passed stools. In no instance was either *S. typhimurium* or *S. typhosa* recovered from the saline controls before or after the saline was subcultured in Selenite-F enrichment medium.

Since stools containing salmonellas might conceivably remain suspended in the saline for periods up to 18 hours, it seemed important to determine whether this prolonged storage might result in an actual decrease in numbers of the salmonellas in positive stools. To clarify this point a suspension of each organism was prepared in exactly the same manner as the cultures employed in the feeding experiments, and sterile flasks containing 3.0 ml. of saline were inoculated with

TABLE 1

Salmonella typhimurium fed to *Blatella germanica*

SEX	DIET*	NUMBER OF BACTERIA FED	NUMBER OF STOOLS PASSED (DAYS INDICATED)	NUMBER OF POS. STOOLS	DAY ON WHICH *Salmonella* ISOLATED FROM	
					STOOLS	Alim. Canal
F	MS	4×10^6	2 (7, 12)	0	—	neg. 26th
M	BHI	4×10^6	1 (5)	1	5th	pos. 10th
M	BHI	5×10^6	2 (2, 7)	2	2nd, 7th	pos. 8th
M	MS	5×10^6	3 (8, 14, 18)	0	—	pos. 18th
M	MS	6×10^6	0	—	—	pos. 2nd
M	BHI	6×10^6	2 (3, 7)	0	—	neg. 14th
M	SM	8×10^6	1 (2)	1	2nd	pos. 11th
F	MS	1×10^7	0	—	—	neg. 6th
F	MS	1×10^7	3 (1, 10, 16)	2	1st, 10th	neg. 26th

* MS—molar sucrose solution; SM—skim milk + 1% yeast extract + 0.5% sucrose; BHI—brain heart infusion broth.

the cultures. Storage was carried out at room temperature and samples of the saline were removed at intervals during a 24 hour period and plated according to standard methods. No apparent decrease in the numbers of viable *S. typhimurium* was recorded, but up to a 45% decrease in *S. typhosa* was observed. Even this mortality would not account for the absence of the typhoid organisms in the many stool cultures previously examined.

In a study to determine the length of time necessary for the ingested typhoid organisms to disappear from the digestive tract of *Blattella germanica*, a group of roaches was fed a heavy suspension of *S. typhosa* in molar sucrose, allowing the insects to feed to satiety. Roaches were sacrificed at three hour intervals, the alimentary canal aseptically removed, macerated, and incubated in Selenite-F broth for 15–18 hours. Isolations of *Salmonella* species were attempted both before and after incubation of the enrichment medium. Positive cultures were obtained at the conclusion of each three hour interval up to and including the twelfth hour. After 12 hours all cultures proved to be negative for the test organisms.

Several *in vitro* experiments were conducted in an attempt to demonstrate some factor responsible for the relatively rapid disappearance of *S. typhosa* in the digestive tract. The alimentary canal of 30 adult untreated roaches was macerated in saline. Loopfuls of this suspension were streaked in parallel lines on the surface of standard agar plates. A fresh broth culture of *S. typhosa* was streaked in lines perpendicular to the suspension streakings, and the plates were then incubated at body temperature for periods up to 48 hours. No inhibition of the test organisms was ever observed. A suspension of the alimentary canal contents was centrifuged and the supernatant was passed through a Seitz filter. The filtrate was tested for antagonistic action against the typhoid organisms using a number of recommended techniques commonly employed with antibiotics, but none of these tests revealed any inhibitory action.

Just what accounts for the disappearance of the salmonellas in the digestive tract of these roaches is not known, and further work is to be conducted in an attempt to explain this action.

SUMMARY

Controlled feeding experiments conducted with adult specimens of *Blattella germanica* mounted on wax blocks are described. Roaches fed an exclusive diet of molar sucrose lived for periods up to 205 days with an average longevity of about 50 days. Diets consisting of beef heart infusion broth or a mixture of skim milk, yeast extract and sucrose were effective in maintaining the test insects over long periods of time.

Prior to feeding the specific *Salmonella* cultures, so-called normal flora studies were carried out on the roaches, and the aerobic and facultative flora are presented. No anaerobic determinations were made.

When *S. typhimurium* was fed to the individual roaches in sufficient numbers, positive stool cultures could be obtained up to the 7th day after feeding the test species, and the alimentary canal contents was found to harbor the viable organisms up to the 11th day after feeding.

Controlled feeding of millions, even billions, of *S. typhosa* to mounted *Blattella germanica* failed to produce a single positive stool after 24 hours in all of the roaches tested, and between 12 and 15 hours after feeding massive doses of the typhoid organism, the alimentary canal contents showed only a single positive culture for this *Salmonella* species. Only two of the 45 stools passed by roaches fed the typhoid organisms were found to be positive. Both of these stools were passed within the first 18 hours after the massive feeding of the test organisms.

None of the tests conducted revealed the presence of any lethal agent responsible for this mass destruction of *S. typhosa*.

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CRITERIA FOR ASSESSING FAT ABSORPTION IN NORMAL SUBJECTS AND SPRUE PATIENTS

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Present knowledge regarding the nature of fecal fat[1] in normal human beings and animals indicates that it is largely of endogenous origin, although dietary and bacterial fat partly contribute to its formation (Sperry, 1927, 1929, 1930, 1932; Angevine, 1929).

Müller's (1884, 1893) early experiments with dogs and professional human fasters demonstrated long ago that stools contained fat even during starvation. His two human subjects eliminated in the neighborhood of 1 gm. of fat per day during a fasting period of about 8 days duration. Later experiments conducted by Hill and Bloor (1922), Sperry and Bloor (1924), and Sperry (1926, 1926a) with fasting animals receiving fat free diets, confirmed Müller's early observations as to a constant endogenous secretion of fat into the intestine. In addition, Sperry and Angevine (1932) working with isolated intestinal loops of dogs kept on a fat free diet, observed that relatively large amounts of fat were secreted into the small intestine and that a considerable portion of this secretion was apparently reabsorbed in the large intestine. The remainder of the unabsorbed fat, together with a relatively small amount of fat secreted into the colon, probably made up the endogenous fat excretion that was found in the feces. By means of fats labeled with deuterium, Shapiro, Koster, Rittenberg and Schoenheimer (1936) showed, that from 65 to 70 per cent of the fat in the diet of humans was absorbed even in the absence of bile and that the majority of the fecal fat originated from fat secreted into the intestinal lumen.

A compilation of the results of fat balance experiments performed in normal human adults by different investigators who utilized easily digested fats at various levels of intake is presented in Table 1. The average fecal fat excretion on very low fat diets, supplying only 8.3 to 9.4 gm. of fat per day was 3.68 $\pm$ 0.73 gm. per day. In the case of the trials in which the experimental subjects received from 40 to 128 gm. of fat per day, the fecal fat excretion was 4.32 $\pm$ 1.78 gm. per day. In the group in which the fat intake per day was 140 to 160 gm., the daily excretion of fat in the stools was 3.88 $\pm$ 1.57 gm.

Statistical analysis of the first three groups indicates that there is no significant difference between the average values of their daily fecal fat excretion. On the other hand, the group which received between 200 and 208 gm. of fat per day gave an average daily fecal output of 8.72 $\pm$ 2.46 gm., a value significantly different from those previously obtained. In addition, Wollaeger and coworkers (1946, 1947) on the basis of their original observations, as well as from data ac-

[1] The term fecal fat as generally used includes, in addition to the true fats, the unsaponifiable matter.

cumulated by Atwater and Benedict, Benedict and Milner, and Wait, plotted a graph showing dietary intake and fecal output of fat. Within the range of 8 to 150 gm. of fat intake, the fat output varied from 3 to 6 gm. per day, values well within the range of those recorded from other sources in Table 1. These results seem to indicate that there is an intestinal threshold for fat, that lies in the neighborhood of 200 gm. per day. When this value is exceeded, dietary fat appears in the feces in larger amounts than usual. It should be noticed that all of these results were obtained from a variety of easily digestible fats of the type found in an average mixed diet. This is important, according to Mattil (1946), because the digestibility, or more correctly, the absorbability of a fat, is inversely proportional to its content of saturated fatty acids, particularly of stearic acid.

It can be thus generalized, on the basis of the data in the literature, that when the dietary fat is low in saturated fatty acids, and the level of fat intake is varied

TABLE 1

Summary of data from the literature on the daily fecal fat excretion of normal adult human beings, arranged in the order of the fat intake

RANGE OF DIETARY FAT INTAKE PER DAY	NUMBER OF OBSERVATIONS	AVERAGE FECAL FAT EXCRETION PER DAY	AVERAGE APPARENT ABSORPTION	AVERAGE CORRECTED ABSORPTION*	REFERENCES
gm.		gm.	%	%	
8.3–9.4	16	3.68 ± 0.73† (2.18–5.60)‡	56.5	100.0	(14, 15, 16)
40–128.3	119	4.32 ± 1.78 (1.8–10.4)	94.8	99.1	(14, 18, 19)
140–168	140	3.88 ± 1.58 (0.8–7.7)	97.5	99.9	(18)
200–208	14	8.72 ± 2.46 (5.5–11.9)	95.8	97.1	(14, 19)

* Average corrected absorption was calculated by subtracting 3.68 g. from the daily excretions and then computing the percentage absorption.

† Standard deviation.

‡ Minimum and maximum values recorded.

within ordinary limits (40–150 gm. per day), the fecal fat excretion, and therefore the percentage fat absorption, are not greatly altered in the average normal adult.

It has been demonstrated by Black, Fourman and Trinder (1946) that sprue patients, when maintained on a low fat diet, excrete the same amount of fat in the stools as do normal subjects. This indicates that the abnormally high content of fat in the stools of sprue patients on a normal ration is largely of dietary origin. As an abnormal loss of fat is considered by many the most constant and characteristic feature of the sprue syndrome, it is therefore important to evaluate the procedures used to detect this abnormality in the stools.

It was the purpose of the present investigation to compare the value of percentage fat in dried feces against percentage fat absorption as an index for assessing fat absorption in normal subjects and sprue patients. In addition, the partition of fat was determined in these experimental subjects.

MATERIAL AND METHODS

In the first part of this investigation a series of 19 normal subjects and 41 sprue patients were studied. In this series the percentage of fat in dried feces and the partition of the same into split fat, neutral fat and unsaponifiable matter were determined. Twenty four hour stools were collected and after thorough mixing in a Waring blender, samples were taken for analysis. The methods of Keye, Leibner and Connor (1940), modified so as to include soap fats by using acidified feces, and the modification of the wet method of Saxon (1914) as worked out by us (1946) were used. Both methods gave similar results. The latter one, however, became the method of choice because it was simpler. Briefly it is as follows: a 3 gm. sample of feces, more or less, containing approximately 10% solids (if they contained more solids the feces were blended with water until the mixture contained about 10% solids) was placed in a graduated centrifuge tube. The sample was acidified with 0.5 ml. of concentrated HCl and then extracted 3 times by shaking with 10 ml. acetone followed by centrifugation. The supernatant acetone extracts were poured into a large graduated cylinder, mixed with 15 ml. of water, and then extracted consecutively three times with 20 ml. volumes of petroleum ether. The fat was recovered by evaporating the petroleum ether. The residue was dried in a desiccator and weighed. The weight of the fat was obtained by difference.

Determinations were made of the percentage of split and neutral fat as well as unsaponifiable matter in the fat residue. This was accomplished by titrating the fat residue, with 0.1 N NaOH until it was alkaline, and then extracting with petroleum ether. The residue left after evaporating the petroleum ether was composed of neutral fat and unsaponifiable matter. This in turn was saponified and then further extracted with petroleum ether. The residue left after evaporating the petroleum ether was unsaponifiable matter. The split and neutral fats were obtained by difference. This method is rapid and gives results comparable to those obtained by other accepted methods. Total solids were determined in a separate sample of feces by drying at 110°C. for 24 hours.

In the second series studied, 7 normal subjects and 7 sprue patients underwent fat balance trials. These trials lasted for at least 3 days, some as long as 17 days. Both groups of persons received the same experimental ration. This ration supplied if fully consumed, in the neighborhood of 90 gm. of fat per day. The items composing the ration were weighed before giving them to the patient. Any residue left was weighed and subtracted from the original weight. All the items that contributed an appreciable amount of fat to the diet were analyzed for their fat content, those that contributed only small amounts were calculated from data given in standard food tables.

Twenty-four hour samples of stools were collected. Before starting and after finishing the trial, the patients were kept on a liquid low-fat diet for a period of 24 hours. The stools collected during the day following the initial low-fat period were discarded. Those collected during the day following the end of the trial completed the collection for the experiment. Although not ideal, this procedure

was better than the use of markers, which has been found very unsatisfactory, especially in the case of sprue patients passing liquid stools. The method used for fecal fat analysis in the second part of the investigation was the modification of the Saxon method already described.

RESULTS AND DISCUSSION

In the first series of 41 sprue patients studied, only 12 showed a percentage of fat in dried feces above 30 per cent. In other words, only 29.2 per cent of the whole group gave a definite indication of steatorrhea according to this procedure. Distribution of the percentage of fat in dried feces in these patients as well as in a series of 19 normal subjects is recorded in Table 2.

TABLE 2

Percentage of fat in dried feces of normal subjects and sprue patients

PERCENTAGE OF FAT IN DRIED FECES	NUMBER OF NORMAL SUBJECTS	NUMBER OF SPRUE PATIENTS	PERCENTAGE OF THE WHOLE GROUP	
			Normal Subjects	Sprue Patients
1–10	1	1	5.3	2.4
10–15	3	6	15.7	14.6
15–20	5	6	26.3	14.6
20–25	3	11	15.7	26.8
25–30	6	5	31.7	12.2
over 30	1	12	5.3	29.2
Total	19	41		

When a normal subject is consuming an average diet, the normal upper limit of fat in dried feces has been considered to be 30 per cent. Although it is evident from the data in Table 2 that in sprue there is a significant increase in the number of cases with more than 30 per cent fat in dried feces, still the number of patients with steatorrhea amounted to less than one third of the whole group studied. This indicates that the determination of percentage of fat in dried feces is not a satisfactory one for evaluating fat loss and fat absorption in normal and, particularly, in abnormal subjects. The principal source of error in this procedure, as an index for assessing fat loss in feces, lies in the fact that the increase in the bulk of stools eliminated, in a condition like sprue, even when expressed on a dried basis, is about the same as the concurrent increase in fecal fat loss. For example, in the second series studied, in which the 24 hours excretion of fecal solids was determined (Table 4), the normal group averaged 25.4 gm. per day and the sprue group 47.5 gm., respectively. As result of this counterbalancing effect, the percentage of fat in dried feces tends to remain within normal limits in the majority of sprue cases. In addition, this procedure does not give any indication of an increased or decreased excretion of fat per unit time because it does not take into consideration the fat balance of the patient.

On the other hand, as pointed out by Black, Bound and Fourman (1947), when

a sprue patient is placed on a controlled diet, and a record is kept of the exact fat intake and of the collection, weight and analysis of all the stools for a definite period of time, it becomes possible to relate fat intake to fat output and the uncorrected or apparent percentage of fat absorbed over the period in question is given by the expression:

$$\frac{\text{dietary fat intake} - \text{fecal fat output}}{\text{dietary fat intake}} 100 = \text{Apparent fat absorption}$$

In the above formula, the metabolic fat, that is, the non-dietary fat excretion, is not taken into consideration. This is permissible, provided fat intake is kept at a level of about 40 gm. per day, since very seldom does the metabolic fat excretion amount to more than 4 gm. of fat per day. If the daily intake of fat is reduced below 40 gm. it is then possible by using the above formula to obtain values for normal subjects below 90 per cent apparent absorption, the minimum value considered normal when using this index. These abnormal values will not be due to poor absorption *per se* but to the fact that the metabolic fat which remains practically constant, becomes a significant percentage of the intake, since the latter has been reduced in amount.

In case it is impossible to keep the patient on an intake of at least 40 gm. of fat per day, a correction should be made in the above formula by subtracting the metabolic fat from the fecal fat output. The remainder is the dietary fat that escapes absorption. The non-dietary or metabolic fat varies within narrow limits between individuals. Never does it amount to 6 gm. per day (see Table 1; group receiving 8.3–9.4 gm. fat per day) and is usually limited to a value in the neighborhood of 4 gm. per day. Obviously it would not be practical to determine this value for each individual patient since this would involve the feeding of unpalatable low fat diets for several days. For this reason, an average figure obtained from normal individuals who have been subjected to a low-fat experiment is usually utilized. As has been pointed out already, the daily fecal fat excretion of normal subjects on low fat diets varies little from that obtained even with a fat intake as high as 150 gm. per day. This further indicates that the average normal subject absorbs well above 90 per cent of its dietary fat intake.

The individual findings of the absorption measurements performed on 7 normal and 7 sprue patients are given in Table 3. The normal subjects consumed an average of 72.6 gm. of fat per day (range 53.4–87.0). Because of the severe anorexia suffered by the sprue patients, the fat intake of this group was only 37.4 gm. per day (range 10.3–68.9). The only patient who consumed over 60 gm. of fat in the sprue group was F.M., an early case with very mild symptoms of the disease. The percentage of solids in the fresh feces averaged 18.6 per cent (range 9.6–27.1) in the normal subjects and in the sprue patients 11.8 per cent (range 5.3–26.1). However, the total quantity of solids passed per day by the sprue patients was roughly twice as much as that passed by the normal subjects. In Table 4 are recorded the individual values for ingestion and excretion of solids as well as the percentage of solids absorbed. This last value was for sprue patients and normal subjects 86.4 and 95.3 per cent respectively.

From the data collected in the fat balance experiment the percentage of fat in dried feces was calculated (see Table 3). The values obtained for the normals was 15.9 per cent (range 8–19.7) and for the sprue, 27.3 per cent (range 14.2–51.0). These results confirmed what has been already stated regarding the percentage fat in dried stools, that is, that it is a very unreliable index for measuring fat absorption because the values obtained from normal, as well as from abnormal cases do overlap a great deal.

TABLE 3

Fat balance experiment (Average values)

CASE	NUMBER OF COLLECTION PERIODS	FAT INGESTED PER DAY g.	SOLIDS IN FRESH FECES %	FAT IN DRIED FECES %	FAT EXCRETED PER DAY g.	FAT ABSORBED	
						Apparent %	Corrected* %
Normal Subjects							
R. B.	3	66.6	19.0	19.2	2.9	95.6	
O. C.	7	71.5	19.7	14.4	2.2	96.9	
M. S.	4	81.9	18.8	17.7	5.6	93.1	
P. V.	4	87.0	10.9	14.1	4.8	94.4	
E. E. J.	4	53.4	27.1	19.7	4.6	91.3	
S. M.	3	64.7	25.3	18.0	5.5	91.6	
J. S.	8	83.0	9.6	8.0	2.1	97.5	
Average		72.6	18.6	15.9	4.0	94.3	
Sprue Patients							
F. M. (early case)	4	68.9	26.1	19.8	5.1	92.7	98.5
E. F.	10	50.0	12.3	40.6	17.9	64.1	72.3
J. M.	17	21.2	10.3	24.1	19.5	8.0	26.9
R. C.	15	20.5	9.1	19.1	6.5	68.4	88.0
C. R.	9	47.3	12.1	51.0	24.4	48.4	56.9
E. V.	3	10.3	7.5	22.3	3.8	63.5	100.0
A. L. R.	5	43.3	5.3	14.2	10.4	76.0	85.3
Average		37.4	11.8	27.3	12.5	60.2	75.4

* Correction consists in subtracting from the average fat excretion per day of the sprue cases the average excretion of the normal subjects, that is, 4 g. per day.

The normal subjects excreted an average of 4.0 gm. of fat per day. The individual values varied between 2.1 and 5.6 gm. The average elimination as well as the range of the individual values was similar to those obtained by other investigators who have performed observations at the same levels of fat intake in normal subjects. The average excretion for the 7 sprue patients was 12.5 gm. (range 3.8–24.4). In a group of 13 recent severe sprue patients reported by Cooke et al. (26), the average daily excretion of fecal fat on an intake of 50 gm. of fat per day was 9.3 gm. (range 3.1–20.8).

The average apparent fat absorption of our sprue patients was 60.2 per cent

(range 8.0–92.7); for the normal group this value was 94.3 per cent (range 91.3–97.5). As has been pointed out, the intake of fat in the sprue patients varied among individuals. Three of these seven patients had fat intakes below 40 gm. per day. It has already been mentioned that under these experimental conditions the results of fat balance studied do not yield apparent absorption figures representative of true utilization, because the metabolic fecal fat is too high in proportion to fat intake. However, if a correction is introduced, as has been already explained, subtracting the metabolic fat, the figure thus obtained is a good index of fat absorption provided, of course, fat intake is not too low, that is, of an order of 10 gm. or less per day. At low levels of fat intake it might happen that the intestinal threshold, although greatly reduced, is still above the level of

TABLE 4

Daily ingestion, excretion and apparent percentage absorption of solids by normal subjects and sprue patients

NORMAL SUBJECTS				SPRUE PATIENTS			
Case	Daily Dietary Solids Ingestion	Daily Fecal Solids Excretion	Apparent Percentage Absorption of Solids	Case	Daily Dietary Solids Ingestion	Daily Fecal Solids Excretion	Apparent Percentage Absorption of Solids
	gm.	gm.			gm.	gm.	
R. B.	461	15.2	96.7	F. M. (early case)	633	26.0	95.9
O. C.	419	15.6	97.0	E. F.	264	44.2	83.3
M. S.	423	31.9	92.5	J. M.	391	80.6	79.4
P. V.	644	34.4	94.7	R. C.	334	44.1	86.8
E. E. J.	466	23.6	95.0	C. R.	295	47.8	83.8
S. M.	710	30.3	95.8	E. V.	225	16.9	92.5
J. S.	600	26.4	95.6	A. L. R.	426	73.0	82.9
Average.....	532	25.4	95.3*		367	47.5	86.4*

* Average of the individual averages.

fat intake. An example will serve as illustration of this situation. If the capacity of the intestine for absorbing fat is reduced 10 times, that is, from a normal threshold of about 200 gm. to a value of 20 gm. per day, it is still possible to obtain a normal fat absorption value provided the fat intake of the subject is reduced below the new threshold of 20 gm. Therefore, on a fat intake of less than 40 gm., corrected fat absorption values of less than 90 per cent can be accepted without question as a reliable indication of poor intestinal absorption, though normal values of 90 or above, are not because the fat intake could be below the reduced intestinal threshold.

In the present trials all the normal subjects exhibited apparent fat absorption values above 90 per cent. These values are a reliable indication of normal intestinal absorption since all these subjects received more than 40 gm. of fat per day. In the case of the sprue patients, 6 exhibited apparent fat absorption below 90. The only exception was patient F. M., an early sprue case, with very mild

clinical symptoms of the disease. His feces contained 25 per cent solids and the apparent and corrected fat absorption values were 92.7 and 98.5 per cent respectively. This case was studied at 3 different levels of fat intake as shown in Table 5.

At a fat intake of 27.1 g. the apparent absorption was only 75 per cent. However, this low value was not due to poor absorption, but to the fact that the metabolic fat was very high in proportion to the fat intake. When the fat intake was increased to 68.9 gm. per day, apparent absorption increased to 92.7 per cent, instead of decreasing as would have been expected in case of poor intestinal absorption. Still when the intake was increased further to 116.9 gm., the apparent absorption remained at the same normal value of 92 per cent. At all the levels of fat intake, the corrected absorption was above 90 per cent. Since this was an early case of sprue, the efficiency of the intestine for absorbing fat was still high enough to maintain normal absorption values, even at the highest level of fat intake.

TABLE 5

F. M. (early case) studied at three levels of fat intake (Average values)

	LOW FAT DIET	NORMAL FAT DIET	HIGH FAT DIET
Number of collection periods	16	10	4
Average total fat ingested per 24 hours, in gm.	27.1	68.9	116.9
Average total fat excreted per 24 hours, in gm.	6.7	5.1	9.4
Average apparent fat absorption, per cent	75.3	92.7	92.0
Average corrected fat absorption, per cent	90.5	98.5	95.5

In patient E. V. apparent fat absorption was 63.5 per cent. However, as the fat ingestion was only 10.3 gm. per day, the apparent absorption value was not reliable. On the other hand, when the value was corrected for metabolic fat the absorption becamn 100 per cent. As previously explained, this figure is also of questionable value, because the intestinal threshold although greatly reduced could be above this very low level of fat intake. It was therefore, impossible, under these conditions, to state with any degree of certainty whether this patient was normal or was suffering from an impairment of fat absorption.

In the sprue group the percentage of fecal fat in dried feces averaged 27.3 (range 14.2–51.0) per cent and the corrected fat absorption averaged 75.4 (range 26.9–100) per cent. However, if case F. M. (early case) and case E. V. (very low fat intake) are eliminated the average corrected fat absorbed becomes much less, 65.9 (range 26.9–88.0) per cent, though the average fat in dried feces remained close to its former value, 29.8 (range 14.2–51.0) per cent. Even after eliminating these 2 cases, the percentage of fat in dried feces was above 30 per cent in only 2 of the 5 remaining patients: case E. F. and C. R. with values of 40.6 and 51.0 per cent, respectively. On the other hand, each of these five patients had a corrected fat absorption value of less than 90 per cent, indicating a significant impairment of fat absorption.

Fecal fat distribution. Table 6 records the results of the fecal fat distribution in the first series of 19 normal subjects and 41 sprue patients and also of the later series of 7 normal subjects and 7 sprue patients. The values for neutral fat were practically the same for both groups. The sprue cases had an average percentage unsaponifiable in the fecal lipids about one half as large as that observed in the normal subjects. This could be due to the increased amount of dietary fat present, which would tend to dilute the percentage of endogenous unsaponifiable matter in the fecal lipids. As regards the sprue patients the average split fat was somewhat higher than in the normals. The presence in the feces of volatile fatty acids arising from carbohydrates fermentation, might explain this increase of split fats in the fecal lipids of the sprue patients.

A disturbing factor, noticed by us as well as other workers (Cooke *et al.*, 1946), is the wide variation in values exhibited between individuals. The figures in

TABLE 6

Distribution as percentage of total fecal lipids

	NUMBER OF CASES	SPLIT FAT*	NEUTRAL FAT	UNSAPONIFIABLE MATTER
		%	%	%
First Series:				
Normal	19	67.9 (52.8–85.3)	15.7 (6.9–28.7)	16.4 (3.8–33.0)
Sprue	41	78.3 (57.3–92.5)	13.9 (3.5–27.5)	8.8 (1.3–28.8)
Second Series:				
Normal	7	63.1 (54.0–81.6)	15.7 (6.1–24.8)	21.1 (11.5–33.2)
Sprue	7	76.2 (62.2–87.2)	14.3 (6.6–21.7)	9.5 (2.5–16.2)

* Split fat = free fatty acids + soap fats.

parentheses in Table 6 indicate the range of values in each series of cases. It seems that other factors in addition to the lipolytic activity of the pancreatic juice do affect in a very pronounced manner the distribution of fecal lipids into split fat, neutral fat, and unsaponifiable matter.

A disturbing factor, noticed by us as well as other workers (26), is the wide variation in values exhibited between individuals. The figures in parentheses in Table 7 indicate the range of values in each series of cases. It seems that other factors in addition to the lipolytic activity of the pancreatic juice do affect in a very pronounced manner the distribution of fecal lipids into split fat, neutral fat, and unsaponifiable matter.

SUMMARY

1. The literature dealing with absorption and excretion of fecal fat by normal subjects and sprue patients is reviewed.

2. In a series of 41 sprue patients and 19 normal subjects studied by us, in which the percentage of fat in dried feces was exclusively determined, only 29.2 per cent of the sprue and 5.3 per cent of the normals had more than 30 per cent fat in the dried feces. The limitations of this procedure for evaluating fat loss in normal subjects and sprue cases is discussed.

3. In another series, percentage fat in dried feces as well as percentage fat absorption, were determined in 7 normal and 7 sprue cases receiving similar care. The percentage of fat in dried feces indicated that 2 out of the 7 sprue cases suffered from abnormal fat absorption. On the other hand, the percentage fat absorption disclosed that 5 out of the 7 patients had definite signs of abnormal fat absorption. One of the 2 remaining sprue cases was a very early case. After repeated studies it always showed normal absorption. The other was a frank clinical case of sprue, which could not be properly evaluated because of low intake of fat.

Daily fecal fat excretion of all the sprue cases averaged 12.5 gm., a value significantly higher than that obtained for the normals. None of the 7 normal subjects treated in a way similar to the sprue patients showed any signs of abnormal fat absorption either when the percentage of fat in dried feces or the percentage of fat absorption was used as an index to assess this condition. The daily fecal fat excretion of the normal group averaged 4 gm.

4. In both sprue and normal subjects the partition of the fecal fat was as follows: the split fat of the sprue group was slightly higher than the normals; the neutral fat was nearly the same as the normals, and the unsaponifiable matter was about half as large as the normals. The possible significance of these results is discussed.

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EMILE BRUMPT[1]
1877–1951

L. W. HACKETT

The Theobald Smith Gold Medal was established in 1935 by the American Academy of Tropical Medicine, to be awarded every other year to an outstanding person who during his lifetime shall have made contributions of the first rank to tropical medicine. The medal itself, with the accompanying certificate of award, has been provided on each previous occasion by George Washington University, but after the 1949 presentation, the University informed the Academy, with regret, that it would be unable to do so any longer. The Academy therefore, at its last annual meeting in Savanna in 1950, resolved, itself, to sponsor the medal, and an anonymous donor has generously provided the funds for making the necessary changes in the medal and certificate and for purchasing the first of the new medals. A committee of three, consisting of Dr. Asa C. Chandler, Dr. Willard H. Wright and myself, was appointed to select the person who should receive the medal in 1951. Thus there will be no interruption in the bestowal of this honor, which in spite of wars and alarms, has been regularly awarded every two years since its establishment.

The names of the seven previous recipients are a sufficient token of the high distinction with which the Academy has intended to clothe this award. The medal has been given in the past to Marshall A. Barber, Richard Pearson Strong, Edward R. Stitt, Charles Franklin Craig, Charles M. Wenyon, Clay G. Huff, and Fred L. Soper. Two of these medallists are in this room today.

The Committee, as it began its deliberations, found itself confronted with a considerable number of highly qualified candidates, among whom it was not easy to choose, but the selection was rendered less difficult by the coincidence that only one name appeared consistently on all of the several lists drawn up independently by the individual members of the Committee. That was the name of Prof. Émile Brumpt of the Faculty of Medicine in Paris, eminent leader of the French School of parasitology and well known and held in affectionate esteem by all the parasitologists and students of tropical medicine the world over. The Committee had no difficulty in making the choice unanimous, and notice of the award was communicated last May to Prof. Brumpt, who expressed his delighted appreciation of the honor but regretted that his physical condition would not permit him to receive the medal in person. Shortly afterwards his health failed rapidly and he died on July 7, 1951, only a few months after the passing of his lifetime collaborators Langeron and Neveu-Lemaire.

Brumpt's genius was extraordinarily vigorous, versatile and competent. An indefatigable traveller, with an insatiable curiosity and unusual powers of observation, every part of the world came to know him well. He lived for his work

[1] Address at the annual meeting of the American Academy of Tropical Medicine, November 16, 1951, on the occasion of the posthumous presentation of the Theobald Smith Gold Medal to Professor Brumpt.

and insisted on seeing everything for himself. As a young man he explored equatorial Africa from the Red Sea to the Atlantic, spending over two years in the jungle, taking over the command of the expedition when his chief died of exhaustion, and bringing back to Europe with him three patients with sleeping sickness, the first ever seen in a European hospital. No difficulties ever deterred him from transporting new parasites to his laboratory in Paris, alive in their natural hosts, from all parts of the world. He travelled from hotel to hotel with cages and crates of snakes, rodents, amphibians, marsupials, birds and even larger animals. I was in Paris once when he arrived from overseas with a cow that was hostess to some peculiar parasite and had to be persuaded to climb the five pairs of stairs to his famous laboratory in the Rue de l'École de Medicine.

I ran across him everywhere in the Far East, in South America, in the Balkans. I spent part of a summer with him in Corsica and we went on several trips together when he was particularly interested in malaria and its vectors. His most valuable, devoted and indispensable assistant was his wife. She went with him everywhere and would take down his observations in the little notebooks which filled a long shelf in his room and to which he could instantly refer when a question arose as to where he had been and what he had seen on some occasion far away and long ago.

His scientific ardor was always leading him into danger; he was ambushed by African warriors, struck by a venomous snake in Venezuela, hospitalized by sunstroke on the Upper Nile and once arrived in New York in coma from a cerebral malaria contracted in Central America. His gravest accident occurred in Paris where he contracted Rocky Mountain spotted fever from material he had brought back with him from a visit to Montana. This was a very serious infection which took him to the threshold of death. For such an active man, his final illness was particularly tragic as his legs became progressively paralysed until he was unable to leave his bed. His mind was unimpaired and when I last saw him in his home last year, he was in constant touch with his laboratory, endeavoring to carry on his work by remote control.

He died in his 75th year, and from the very beginning of his scientific career, and in every field of parasitology, he was constantly contributing something very substantial and permanently valuable to our knowledge. In Brazil he suggested xenodiagnosis in Chagas disease; on a brief visit to Colombia he described seven new species of Phlebotomus; in Ceylon he discovered *Plasmodium gallinaceum*, cause of the malaria of fowls and permitting a very important advance in our penetration of the secret of the sporozoite cycle in the vertebrate host.

This is not the place to attempt an account of Brumpt's scientific accomplishments. They are set forth in the more than 400 publications from his pen, but the greatest monument to the universality of his interests and knowledge is his fine book, the "Précis de Parasitologie". When the first edition appeared in 1910, no similar work existed anywhere which brought together such a wealth of documentation, so carefully illustrated. As edition followed edition, it had an enormous prestige and influence, and Brumpt took the greatest pains to keep it abreast of the times, so that finally with the growth of knowledge it

was forced to undergo binary fission and appear as two volumes of equal size, a process of division carefully supervised by Brumpt from the bed on which he now lay semi-paralysed.

But perhaps his finest and more effective work was as a teacher. Blanchard's old laboratory was transformed by Brumpt's eager vitality and incredible devotion to his work and to his students into a powerful, adaptive and living instrumentality for scientific investigation and instruction in parasitology. Every year he personally garnered fresh material, living rather than formolized, from the four corners of the earth, and annually professors and students alike gravitated to his laboratory from everywhere to observe and experience the magic touch.

I have wanted to present to a younger generation interested in tropical medicine, this rapid sketch of the man who was possibly the last great general parasitologist; who succeeded, without falling into superficiality or wasteful dispersal of effort, in cultivating some important part of every field of parasitology.

Posthumously though it has to be, it is an honor to present to you, M. de Moras, on behalf of the memory and the impressive scientific legacy of Émile Brumpt, the Theobald Smith Gold Medal of the American Academy of Tropical Medicine.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

Rosenau's Preventive Medicine and Hygiene. By Kenneth F. Maxcy, Professor of Epidemiology, Johns Hopkins University School of Hygiene and Public Health, with 26 contributors. 7th edition. 1,462 pp., 131 illustrations, frontispiece in color. New York, Appleton-Century-Crofts, Inc., 1951. Price $14.00.

Tropical Nutrition and Dietetics. By Lucius Nicholls, M.D., Director, Division of Nutrition, Ceylon; Nutrition Adviser to Commissioner-General, South East Asia. 3d edition. 476 pp., illustrated. London, Ballière, Tindall & Cox; Baltimore, The Williams & Wilkins Co., 1951. Price $6.00, 42/.

Biological Antagonism. The Theory of Biological Relativity. By Gustav J. Martin, Sc.D., Research Director, The National Drug Co., Philadelphia. 516 pp., 64 figures. Philadelphia, The Blakiston Co., 1951. Price $8.50.

Yellow Fever in Galveston, Republic of Texas, 1839. An Account of the Great Epidemic. By Ashbel Smith, M.D., ex-Surgeon General of the Texian Army, together with a biographical sketch by Chauncy D. Leake, and stories of the men who conquered yellow fever. 135 pp., illustrated. Austin, University of Texas Press, 1951. Price $2.50.

Pharmacopoea Internationalis, Volume I. Ed. World Health Organization. First edition, 406 pp., cloth. Geneva, Switzerland, World Health Organization, 1951. Price $5.00, 35/-, Sw.fr. 20.00.

The Battle for Mental Health. By James Clark Moloney, M.D. 105 pp. New York, The Philosophical Library, 1952. Price $3.50.

A Manual of Parasitology for Medical Students and Beginners. By Mark M. Schapiro, M.D., with foreword by Sir Philip Manson-Bahr. 140 pp., 8 plates, with frontispiece in color. Cloth. New York, Greene and Stratton, Inc., 1951. Price $5.50.

Industry and Tropical Health, proceedings of the First Industrial Tropical Health Conference, Sponsored by The Harvard School of Public Health, December 8–10, 1950 at Boston, Mass. Boston, Published by the Harvard School of Public Health for the Industrial Council for Tropical Health, 1952. Price $10.00.

Introduction to Malaria Problem in India. By Lt. Col. B. L. Raina, Army Medical Corps, with an introduction by Dr. Jivraj N. Mehta. 131 pp., illustrated. Bombay, New Book Co. Ltd., 1951. Price Rupees 7/12.

The Cost of Sickness and the Price of Health. By C.-E. A. Winslow, Dr.P.H., Consultant in Public Health Administration, W.H.O., Professor Emeritus of Public Health, Yale University. 106 pp. World Health Organization: Monograph Series No. 7 (Available also in French), 1951. Price $1.50; 7s.6d.; Sw.fr. 6.00.

Diseases in Old Age, a clinical and pathological study of 7941 individuals over 61 years of age. By Robert T. Monroe, M.D., of the Peter Bent Brigham Hospital and the Dept. of Medicine, Harvard Medical School, Boston, Mass. 407 pp. Cambridge, Massachusetts, Harvard University Press, 1951. Price $5.00.

Essentials of Histology. By Margaret M. Hoskins, Ph.D., and Gerrit Bevelander, Ph.D., New York University. 2d edition, 240 pp., 135 illustrations, 2 in color. St. Louis, The C. V. Mosby Company, 1952. Price $4.00.

BOOK REVIEWS

Clinical Tropical Medicine. Edited by R. B. H. Gradwohl, M.D., Luis Benitez Soto, M.D., and Oscar Felsenfeld, M.D., with 57 contributors. 1647 pp., illustrated. St. Louis, The C. V. Mosby Company, 1951. Price $22.50.

During the past few years, apparently as a result of interest stimulated in tropical medicine during World War II, several new texts have appeared, including the present one under review. It is questionable whether any great contribution is made by attempting to coordinate the contents of texts dealing with parasitology, mycology, clinical tropical medicine and entomology in a single overly large book. This new text is the joint work of 57 contributors and three editors. Some of the chapters, notably those dealing with the helminths and local conditions in Central and South America, are extremely well presented. On the other hand, relatively more important subjects, such as malaria and visceral leishmaniasis, receive casual treatment. There seems to be a disproportion in this respect from the standpoint of local distribution and human suffering and disease in the space allotted for the various subjects. For example, the subject of trichinosis, while extremely well presented, is given more space than malaria. The illustrations, dealing with the pathology of many infections, notably those produced by the protozoa, are extremely poor. In many instances, while treatment of a given infection is fairly inclusive, no critical evaluation of drugs is made, and no specific recommendations are made to the reader, who may not be in a position to choose the most effective drugs. In the opinion of this reviewer, the text may be useful for reference to certain chapters, but it is doubtful that a text of this size and scope represents a major contribution to existing texts and systems of tropical medicine.

Harry Most

Lutte Antipaludique par les Insecticides a Action Rémanente, Resultats des grandes campagnes, by E. J. Pampana, Chief, Malaria Section, W.H.O., pp. 72, Geneva, Switzerland, World Health Organization, 1951, price 5/—, $1.00, Sw. fr. 4.00.

This is an orderly summary of information concerning the major malaria control programs in various countries of Africa, the Americas, Asia, and Europe where residual insecticides have been used. Where possible, the author has recorded the size of the population exposed to malaria; the area of its endemicity; names of the important vectoral anophelines; the type, frequency, and application rate of the formulation used; the numbers of premises sprayed and of persons protected, the unit cost, and the results obtained. These last are expressed in terms of malaria morbidity and mortality, and of other paludometric data where available before and during the antimalarial campaigns. Much of the information is shown graphically in 11 figures, and statistically in 22 tables. Some 50 bibliographic references are cited, though it is evident that a considerable portion of the content has been derived from the personal communications of Dr. Pampana with malariologists in various countries.

While it has not been possible to include reports from all areas where residual insecticides are being used on a large scale, this review is sufficiently panoramic to portray an authentic picture of world-wide malaria recession correlated with the use of residual mosquito-killing chemicals.

This resume will undoubtedly be of great technical interest to the public health administrators, medical historians, and others concerned in global disease trends and control accomplishment. It should constitute an impressive and convincing lesson in the possibilities of wholesale communicable disease prevention—perhaps even eradication!

This small volume is written in French but contains an excellent summary of essential content in English.

Justin M. Andrews

Clinical Laboratory Methods, Fourth edition, by W. E. Bray, M.D., Professor of Clinical Pathology, University of Virginia. 614 pages, with 119 text illustrations and 18 color plates. St. Louis, C. V. Mosby Company, 1951. Price $7.50.

The new fourth edition of this well known book follows the same plan and in general covers the same fields as the former edition. It has been enlarged, however, to the extent of about 100 pages by the inclusion of much new material, and it has been brought well up to date.

In addition to the usual microscopic and chemical procedures which would be anticipated in a book on Clinical Pathology, there is an ample section on Bacteriology and Mycology. Hematology is well covered, and much has been added regarding the blood groups and problems concerning the Rh factor. Much has also been added to the sections on Blood Chemistry, including tests for many extraneous drugs and poisons.

The text is supplemented with many tables, graphs and illustrations, including 18 colored plates.

The portions dealing directly with Parasitology will have little of interest to those specializing in this field, although adequate for those for whom the book is intended.

The book is an excellent compendium of clinical laboratory procedures, with sufficient background to make clear the indications for and significance of such procedures. As such it should prove very useful and is recommended.

Paul W. Clough

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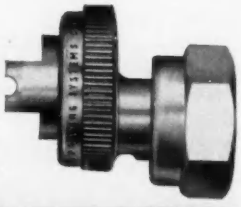

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Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

Authors are asked to cooperate by complying with certain standard practices in the preparation of their manuscripts in order to facilitate the editorial task:

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Tables should be typed on separate sheets and be serially numbered. Glossy prints of tabular material eliminate expensive typesetting.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

1. Snyder, T., and Meleney, H. 1941, The excystation of *Endamoeba histolytica* in bacteriologically sterile media, *Am. J. Trop. Med.* **21:** 63–73.

Citation in the text should be by author and date.

Proprietary names of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U.S. Pharmacopoeia, New and Nonofficial Remedies of the A.M.A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

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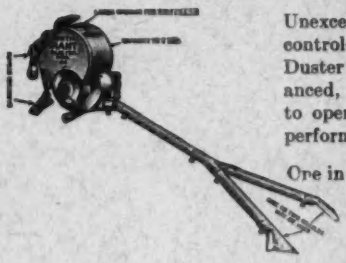

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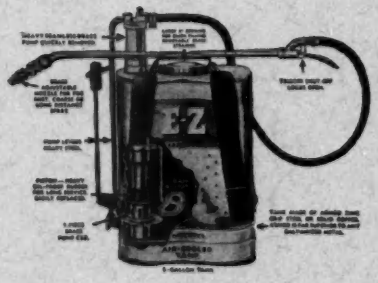

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